U.PORTO

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

MSc

2.º CICLO

FCUP

2018

Forensic DNA mixtures: Analysis and comparison of software results

Carolina Marques Ferreira Figueiredo

Forensic DNA mixtures: Analysis and comparison of software results

Carolina Marques Ferreira Figueiredo

Dissertação de Mestrado apresentada à
Faculdade de Ciências da Universidade do Porto em
Genética Forense

2018

Forensic DNA mixtures: Analysis and comparison of software results

Carolina Marques Ferreira Figueiredo
Mestrado em Genética Forense
Departamento de Biologia
2018

Orientador
Nádia Maria Gonçalves de Almeida Pinto, Instituto de Investigação e Inovação em Saúde (i3s), Instituto de Patologia e Imunologia da Universidade do Porto (IPATIMUP), Centro de Matemática da Universidade do Porto (CMUP),
Faculdade de Ciências e Tecnologias da Universidade do Porto (FCUP)

Coorientador
Paulo Miguel Ferreira, Especialista Superior,
Laboratório de Polícia Científica da Polícia Judiciária (LPC-PJ)

U.PORTO

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, _____/_____/_____

Acknowledgments

For having contributed to this work in some way, I would like to express my gratitude to:

My supervisor Nádia Pinto, for all the guidance, help and support which were crucial to finish this work. All the exchange of ideas contributed greatly to it and I deeply appreciate all the dedication.

My external supervisor, Paulo Miguel Ferreira, for all the practical knowledge transmitted that was hugely important not only to the development of this work but also to my future. Also, for open me the doors to new experiences.

Every single member of the sector Biology, a huge thank you for making me feel so welcome and for all the wisdom shared. I truly thank the opportunity given for the internship in LPC-PJ.

Eduardo Conde-Sousa, for the willingness to provide an important contribution for the development of this work.

My colleagues, with whom I shared this journey. It becomes less hard when we are not alone in it.

My great friends, Inês Carneiro, Diana Vieira, Cátia Gomes, Joana Brito, Inês Martins, Diogo Gama. Our encouragement for each other really is comforting and unwinding in the middle of stressful times. Thank you for being present, even far.

Ricardo, a big thank you for all the total support and for the motivation in all steps of the way.

Finally, to my parents, for always support my decisions, unconditionally. My accomplishments are thanks to you.

Abstract

Forensic samples recovered from crime scenes often contain genetic material from more than one contributor, originating profiles with multiple alleles per *locus*. Also, these samples are frequently composed by DNA in low quantity and/or quality, which favors the occurrence of stochastic effects, like drop-in and/or drop-out. Adding the possible presence of artifacts in an electropherogram, like stutter peaks, the outcome can be a very complex interpretation.

The number of contributors of DNA to a mixture can only be estimated, typically through the observation of the number of alleles per marker and peak imbalance. However, the mentioned effects and allele sharing between contributors (masking effect), can lead to a wrong estimation.

Mainly when dealing with complex samples, it is important to quantify its probative value through the computation of the Likelihood Ratio (LR), which compares the probabilities of observing the evidence assuming two opposite hypotheses. Several computer programs based on the LR approach have arisen, differing on the applied probabilistic methods. These programs are typically divided into those which are based on a) qualitative models, that only use qualitative information of the electropherogram; and b) quantitative models, that also use quantitative information (peak heights).

In this work, we recovered real casework mixture profiles (of two and three estimated contributors) from former cases of the Laboratório de Polícia Científica da Polícia Judiciária (LPC-PJ), as well as its respective reference profiles. Also, we simulated profiles of relatives of the casework references – one full-sibling and one parent. To each of the references (casework and simulated), we perform identity tests computing a LR (with the hypotheses of the reference being a contributor to the mixture and being genetically unrelated to any contributor of the mixture) using the estimated number of contributors and varying it by under- and overestimation. Moreover, we observed the impact on the LR when varying other parameters considered by the software, related to the co-ancestry coefficient of the population, allele drop-in and detection threshold limit, using the casework references. All the analyses were performed resorting to a quantitative software (Euroformix) and a qualitative one (LRmix Studio). The obtained LRs were also compared in an inter-software analysis.

The computed LRs through the different approaches diverged, producing the quantitative model higher LRs. Generally, the parameters' variation and the change of the estimated number of contributors had little effect on the LR. Notwithstanding, in some cases, the LR was greatly affected, specifically when the number of contributors was underestimated.

The results reinforce the importance of a cautious electropherogram interpretation and statistical analysis in order to obtain a reliable weight of the genetic evidence.
Keywords: Forensic casework; DNA mixture; STR profile; Likelihood Ratio; Software

Resumo

Amostras forenses recolhidas em local de crime muitas vezes contêm material genético proveniente de mais do que um contribuidor, originando perfis com múltiplos alelos por *locus*. Estas amostras são, frequentemente, compostas por ADN em baixa quantidade e/ou qualidade, o que favorece a ocorrência de efeitos estocásticos, como *drop-in* e/ou *drop-out*. Adicionando a possível presença de artefactos num electroferograma, como picos *stutter*, a interpretação pode-se tornar bastante complexa, consequentemente. O número de pessoas que contribuíram com ADN a uma mistura apenas pode ser estimado, o que normalmente é conseguido através da observação do número de alelos por marcador e pelo balanço de massas. Contudo, os efeitos mencionados e a partilha de alelos entre contribuidores (*masking effect*), podem levar a uma estimativa errada.

Principalmente lidando com amostras complexas, é importante quantificar o seu valor probativo através de uma Razão de Verosimilhança (LR, do inglês *Likelihood Ratio*), que compara as probabilidades de observar a prova segundo duas hipóteses opostas.

Vários programas de computador baseados na abordagem do cálculo de LR surgiram, diferindo no método probabilístico aplicado. Estes programas são tipicamente divididos naqueles que se baseiam em a) modelos qualitativos, que apenas utilizam informação qualitativo do electroferograma; e em b) modelos quantitativos que também utilizam informação quantitativa (alturas dos picos).

Neste trabalho, recuperámos perfis de mistura (de dois e três contribuidores estimados) de antigos casos reais do Laboratório de Polícia Científica da Polícia Judiciária (LPC-PJ), assim como os respetivos perfis referência. Também simulámos perfis de parentes das referências dos casos reais – um irmão e um pai. Para cada uma das referências (dos casos reais e simuladas), foram efetuados testes de identidade, calculando um LR (com as hipóteses da referência pertencer à mistura e de ser geneticamente não relacionada com nenhum contribuidor da mistura) usando o número de contribuidores estimado e variando-o, sub- e sobrestimando-o. Adicionalmente, observámos o impacto no LR ao variar outros parâmetros considerados pelos *software*, relacionados com o coeficiente de co-ancestralidade da população, *drop-in* e limite de deteção, para as referências reais.

Todas as análises foram realizadas recorrendo a um *software* quantitativo (*Euroformix*) e a um qualitativo (*LRmix Studio*). Os LRs obtidos foram também comparados numa análise inter-*software*.

Os LR calculados através das diferentes abordagens divergiram, sendo que o modelo quantitativo produziu LRs mais elevados. Globalmente, a variação dos parâmetros e a alteração do número de contribuidores estimado teve pouco efeito no LR. Contudo, nalguns casos, o LR foi fortemente afetado, concretamente quando o número de contribuidores fui subestimado. Os resultados reforçam a importância de uma interpretação do electroferograma e análise estatística cuidadas e atentas, de forma a obter um valor probatório fiável.

Palavras-chave: Forense; Misturas de ADN; Perfil de STRs; *Likelihood Ratio*; *Software*

Contents

List of Tables

List of Figures

Abbreviations

DNA – Deoxyribonucleic acid

VNTR – Variable Number Tandem Repeat

bp – Base Pair

STR – Short Tandem Repeat

SNP – Single Nucleotide Polymorphism

InDel – Insertion or Deletion

RFLP – Restriction Fragment Length Polymorphism

PCR – Polymerase Chain Reaction

SDS - Sodium Dodecyl Sulfate

DTT – Dithiothreitol

epg - Electropherogram

qPCR – Quantitative PCR

C_T – Cycle Threshold

IPC – Internal PCR Control

dNTP - Deoxynucleotide Triphosphate

CE – Capillary Electrophoresis

RFU – Relative Fluorescent Unit

LT-DNA – Low Template DNA

LCN – Low Copy Number

ISFG – International Society of Forensic Genetics

MAC – Maximum Allele Count

POI – Person of Interest

LR – Likelihood Ratio

RMNE – Random Man Not Excluded

F_{ST} – Co-ancestry Coefficient

IBD – Identical by Descendent

T – Threshold Limit

LPC-PJ – Laboratório de Polícia Científica da Polícia Judiciária

NIST - National Institute of Standards and Technology

1. Introduction

1.1. DNA structure and organization

The deoxyribonucleic acid (DNA) is a double stranded molecule arranged in helical form, discovered in 1953 by Watson and Crick [1], localized in the nucleus of the cells. It is formed by nucleotides units, which comprises a triphosphate group, a deoxyribose sugar and a nitrogenous base - adenine, cytosine, thymine or guanine. These are complementary in a specific way: adenine only pairs with thymine and cytosine with guanine; hydrogen bonds between the bases sustain the double strand conformation [1]. Humans have approximately three billion base pairs [2]; each of the nitrogenous bases provides the variation in nucleotides, since it is the variable element. Its immense possible sequence yields the biological diversity among living beings [3]. Concerning to the human beings, in some regions, the DNA sequence is the same to all the individuals of the specie and, in other regions, different; some of these differences are responsible for the distinct physical features of each individual.

This nucleic acid codes the information needed to accomplish its purpose: replicate itself so that all cells of the individual carry the same genetic material and synthetize proteins required for cell functions [3].

The human nuclear DNA is condensed and organized in 23 pairs of chromosomes (22 autosomal, i.e. similar in both females and males, and one sex-determining); each of the chromosomes of a pair is inherited from each parent (although they do not comprise exactly the same genetic information due to an exchange of information between the chromosomes of the parents - crossing over, during meiosis). These organization structures are contained in the nucleus of the cells and the entire genetic information of a cell is called the genome.

The human genome was studied through the Human Genome Project, that sequenced 99% of the euchromatic human DNA [4].

Based on the structure and function of different regions of the DNA, it can be divided in different groups. Most of the DNA does not code the synthesis of proteins, being either extragenic regions or introns (within the genes). The polymorphic DNA markers used for forensic purposes are required to be located in these non-coding regions [5].

1.2.Types of genetic polymorphisms

Except identical twins (barring somatic mutations), it is expected that all individuals have different DNA and so, although it is estimated that only 0.3% of our DNA is variable, the probability of two individuals share the same DNA profile is virtually zero, for recombining markers [3; 6]. Recombination happens in autosomal and X-chromosomal markers in each generation, shuffling the genetic information and this way contributing to human diversity [5; 6]. The existing diversity in variable regions of autosomes makes it useful for forensic matters regarding to human identity, i.e., determining if there is a match or not between two samples [3], and other kinship problems. Due to the work developed on the human DNA, specific locations in those regions better suited for the mentioned purposes are now known (ex: markers with higher mutation rates).

Genetic variation can be seen in the form of sequence or length polymorphisms.

1.2.1. Minisatellites or Variable number tandem repeats (VNTRs)

Minisatellites or Variable number tandem repeats (VNTRs) are length polymorphisms consisting on a sequence being repeated in tandem in a variable number of times among different genome locations and also among individuals – reason why it is possible to differentiate persons with this type of markers.

The size of the repetitive motif of this polymorphism ranges from six to 100 bp (base pairs), which can be repeated thousands of times [7].

These were the first markers used in forensic genetics casework [8]. However, its use was limited by the high quantity of DNA required to the analysis and by the difficult interpretation of the results obtained, being their use in forensics replaced by other type of polymorphisms [2].

1.2.2. Microsatellites or Short Tandem repeats (STRs)

Microsatellites or Short Tandem repeats (STRs) are polymorphisms which also vary in length, distinguishable from the previously described by the smaller size of the repeated sequence, as the name indicates, ranging from one to six bp, being the most common in forensic use tetranucleotide repeats [2]. As in VNTRs, the variable number of repetitions is

what differentiate individuals, with the distinction of smaller repetition numbers in this case. This variation is generated by random mutations, in which they gain or lose repeats by replication slippage [9].

Due to its characteristics, STRs become the widely used type of markers in forensics: (a.) abundant in the nuclear genome (mainly in non-coding regions [10]); (b.) high mutation rate – ranging between 10^{-3} and 10^{-4} [11] - and consequently a high intrapopulational diversity (i.e., they are highly polymorphic since there are various allelic possibilities for a locus); (c.) low interpopulational diversity [10], which allows for not so distinct populational allele frequencies; (d.) it can be amplified in one multiplex (amplification of various loci in a single reaction), diminishing possible human errors and contamination;(e.) its processing can be automated, turning it simple and fast; (f.) the obtained results are easy to interpret since it consists on discrete alleles;(g.) it is possible to amplify STRs with low quantities of DNA and even with degraded DNA [12].

Due to the general use of this type of markers, soon began to appear commercial kits to type STRs, which improved the interlaboratory consistency.

1.2.3. Single nucleotide polymorphisms (SNPs)

SNPs are sequence polymorphisms in which, as the name indicates, a single nucleotide is substituted in a certain DNA sequence, through mutation occurrence during DNA replication in meiosis. It is the most abundant type of variation in the human genome: comparing a typical genome to the reference human genome, it was found that circa 96% of the variants consist of SNPs [13]. Because SNPs are typically biallelic (i.e., two possible bases for the respective nucleotide), these variable portions are not so polymorphic and, consequently, not so informative as STRs. To make them more discriminating it would be necessary to examine a large amount of them [14]. Particularly regarding to mixtures, the use of SNP markers would be problematic due to its only two allelic possibilities. On top of that, the processing is not as simple and rapid as the processing of STRs [2].

Despite such limitation, SNPs can be an option in cases involving degraded DNA, due to its small amplicons [15]. In addition, SNPs can be used to provide information on kinship analysis [16] (despite care should be taken when close relatives are involved [17]) and on geographic ancestry [18], considering its low mutation rate of the magnitude of 10^{-8} [19].

1.2.4. Insertions and deletions (Indels)

Insertions and deletions (Indels) are length polymorphisms which are characterized by the insertion or deletion of one or more nucleotides in the genome. They are fairly common in our genome, representing about 4% of the variants detected comparing a typical human genome to the reference one [13]

Its mutation rate is also low – order of magnitude of 10^{-8} [19], so they are not as polymorphic as STRs and, consequently, not as discriminating for individual identification. On the other hand, Indels can also be informative about populational studies and geographic ancestry [20, 21]. Small sized Indels allow for a short amplicon analysis, which is useful in cases with degraded DNA. Moreover, its processing can be simple as the processing of STRs [22].

1.3. Historical context of Forensic Genetics

In 1900, Karl Landsteiner observed that individuals could be placed in different groups based on their blood types, describing the ABO blood system. It was the first tool used in forensic matters, when in 1915 a paternity case was solved resorting to this system. Henceforth, other blood group markers were used in forensic laboratories, as well as protein profiling through gel electrophoresis. Despite the low discriminating power of these methods, they were capable of exclude individuals when reference and problem profiles did not match [3].

It was in 1985 that Alec Jeffreys realized the potential of hypervariable regions of genetic material to be applied to human identification, calling it “DNA fingerprint” [23, 24]. After digest human DNA with a restriction enzyme, he separated the fragments by agarose electrophoresis, transferred it to a nitrocellulose membrane and subjected it to hybridization with labeled probes complementary to minisatellites and flanking regions. The length polymorphism shown by these repetitive regions in DNA from different origins allowed him to infer that they could be used to specifically identify individuals. This method was applied for the first time in an immigration case, in the same year [25]. In 1987, the DNA fingerprinting was firstly successfully used in a criminal case [26].

Which takes us to the definition of forensic genetics. A descriptive one, used by the “Forensic Science International: Genetics” Journal is: “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". As so, DNA is currently used worldwide as a crucial tool in civil and criminal cases through kinship testing (identification included). In this work the focus will be the criminal application of identity testing, considering biological material containing DNA to link a perpetrator to a crime scene.

In the 1990s, methods and techniques quite evolved from the one previously described, as well as the types of DNA polymorphisms analyzed. Methods based in Restriction Fragment Length Polymorphism (RFLP) had some limitations concerning to quality and quantity of DNA, besides the difficult comparison between genetic profiles, being replaced by more sensitive and fast methods based on Polymerase Chain Reaction (PCR) [26, 27]. Initially, the polymorphisms used in PCR based systems were SNPs, which substituted the use of VNTRs; afterwards, STRs became the most used DNA polymorphisms in forensic genetics, due to their great discriminating power [26, 28]. Around the change of the millennium, the first widely used commercial PCR kits to type multiple STRs arose, but with a limited number of markers [29]. Since then, the number of loci targeted in a multiplex reaction had been increasing and, currently, these kits are composed by more than 20 STRs, also increasing the ability to discriminate [30]. The set of STRs composing the current multiplex typing kits have an extremely low random match probability (chance of two random, unrelated, individuals share the same profile) [31].

These advances allowed for minimal quantities of (even degraded) DNA to be analyzed in an automated process, in short time and providing very informative data.

1.4. Forensic samples processing

1.4.1. Collection

In almost every criminal case, there is biological material left behind by the victim and/or perpetrator. After collection of the material, it is possible to obtain cells and, consequently, DNA. With the introduction of PCR, the ability to obtain a genetic profile through small quantities of biological material improved, since it became possible the amplification of specific DNA fragments. This increased sensibility can represent, however, a potential disadvantage. Indeed, it is required an extremely cautious collection and handling of the material in order to prevent contaminations of the evidential genetic material with DNA from a source extra to the crime scene, like from a crime scene officer, and possible wasting an important evidence to the investigation. Likewise, the preservation process must be done

correctly by means of maintain a chain of custody so that the evidences can have value in court.

A wide variety of evidences can be collected from a crime scene to potentially extract DNA from it in the laboratory. Some of those items may be the weapon of the crime, clothes, shoes, balaclavas, cigarette butts, swab of a steering wheel and others. Commonly analyzed biological materials are blood, semen, rooted hair and epithelial cells from the skin.

While some biological stains are easily visible, other can be a little more challenging to detect or identify. Alternate light sources proved to be a helpful method of detection and/or identification of biological stains, since through emission of light in different wavelengths, biological fluids like semen, saliva and blood fluoresce [32]. Several rapid presumptive tests can also be used for identification of body fluids, mainly blood (most of these relying in the peroxidase-like activity of hemoglobin) [33, 34]. In addition, there are other type of techniques for identification of the origin of a biological material using profiling of mRNA, microRNA or DNA methylation [35-37].

So that it is possible to identify the origin of the DNA profiles obtained in the recovered evidences from the crime scene. Reference samples must also be collected to be compared. These are collected from the victim and the suspect(s), usually by buccal swab, yielding a single source, theoretically optimal, DNA profile.

1.4.2. DNA Extraction

To isolate the intended molecule – the DNA – it is necessary to extract it from inside the cells of the biological sample and separate them from other cellular components.

The extraction process can rely on different types of techniques, like organic extraction, Chelex extraction or solid-phase extraction.

The first typically uses a detergent (sodium dodecyl sulfate - SDS) and proteinase K to cause cell lysis and phenol-chloroform to denature the proteins. After centrifugation, an organic and an aqueous phases are formed, the latter containing the nucleic acids. The DNA is purified from this phase by ethanol precipitation or filter centrifugation [38]. This method was widely used but fall into disuse due to the toxicity of phenol. Another disadvantage was the multiple tube changes required that increased the possibility of contamination and make the process more laborious [2].

Chelex extraction is based on the use of a resin, with the name of the method, in the form of beads that are added to the sample as a suspension. The mixture is boiled so that the cell membranes disrupt, as well as cell proteins. Chelex has a very high affinity to polyvalent metal

ions, such as magnesium, being, therefore, chelated. Magnesium can act as catalyst in DNA degradation; hence, by removing it, the DNA molecules are protected. After centrifugation, a supernatant with the DNA in single strand is obtained [39]. This is a rapid, low-cost and simple method, with diminished possibility of contamination [2].

FTA® paper was developed as a way of collect and store DNA samples, particularly blood. This paper is impregnated with denaturing chemicals that also protect and preserve the DNA, inhibiting degradation by nucleases and micro-organisms growth, allowing for the stability of the DNA for several months. So, when in contact with the paper, cell lyses and the DNA bounds to it. To purify the DNA, a small portion of the paper is punched and placed onto a tube and non-DNA components are washed off. The punched paper, now with purified DNA, is then directly subjected to PCR [40]. The major disadvantage and reason why it is not widely currently used, is because the dry piece of cut papers can move between wells in a sample tray due to static electricity [3].

Nevertheless, as in many other processes, laboratories have the need to automate. There are several systems that allow so, mainly solid-phase extractions, which relies on the selective bound of DNA to a solid substrate (silica, glass, magnetic beads) [41]. Currently in use in forensic laboratories are the commercial kits developed based on this type of extraction, such as PrepFiler™ Forensic DNA Extraction, which is a magnetic particle-based DNA extraction system. Initially, the piece of evidence is placed into a filter column, that goes into a spin tube. Then, a pre-processing stage is required: after the addition of a lysis buffer, dithiothreitol (DTT) and, in some kits, Proteinase K to the sample, the tubes are placed in a thermal shaker and then centrifuged. At this point, the samples lysate containing the genetic material are collected in the spin tube and the column is discarded. Henceforth, the remaining extraction process can be automatically completed in an equipment, since the kit also has cartridges with different compartments with the required reagents, including the magnetic particles [42].

Different types of kits can be used to deal with optimal, single source samples (reference samples), such as SwabSolution™ kit.

A particular case of extraction is the so-called differential extraction. This type of extraction is performed to separate female and male fractions of a mixed sample, generally in sexual assault crimes. The biological samples recovered in this type of crime usually contains epithelial cells originated from the female victim along with the spermatic cells from the male perpetrator (considering a typical case of this nature). The techniques used to separate the two distinct types of cells are based on the method described in 1985 by Peter Gill and colleagues [43]. The male DNA present in the spermatozoa is quite protected (by the acrosome, that encapsulates the nucleus). Thus, this is the base for the selective extraction:

the male fraction is extracted with a more aggressive technique, while the female epithelial cells can be lysed with a mild treatment. After the female cell lyses with a detergent (like SDS) and a proteinase (usually proteinase K), they are centrifuged and removed to a different tube to isolate the female fraction; the initial sample continues the extraction with the addition of DTT, to help the release of the male genetic material [43].

1.4.3. DNA Quantification

Before amplification, it is important to determine the amount of DNA present in the extracts, so that the quantity included into the PCR reaction be appropriate to yield a good quality electropherogram (epg). Too much or not enough DNA will result in a profile difficult to interpret. To ensure a good result, the samples may be adjusted by dilution or concentration.

Because reference samples are, in theory, optimal samples, they are not usually quantified. Contrary to current methods, in an early period, quantifications were not species-selective, as they quantified the total DNA present in an extract, i.e., besides the human DNA, non-human DNA (from plant, animal, bacteria) that could be present were quantified as well. Ultraviolet and fluorescent spectroscopy and gel electrophoresis-based analysis were initially performed to quantify DNA. However, they had the disadvantages of low specificity or sensibility [44].

To overcome the problem of low specificity, two methods had arisen: hybridization by slot blot using a primate-specific probe [45], and a system of detection using *Alu* repeats, which are specific and abundant in the human genome [46]. However, these procedures were very laborious and the sensitivity had room to improve [44].

Real-time PCR or quantitative PCR (qPCR) was described in the early 1990s [47, 48] and has been widely used in different assays to accomplish not only the purpose of quantification, but others (like indication of the level of DNA degradation of a sample) [49]. The most common approach to the technique uses a TaqMan probe, which is labeled with two molecules - the reporter fluorophore and the quencher (which suppresses the emission of fluorescence by the reporter when they are close to each other). The probe is complementary with the amplicon sequence (between the two primers region), hybridizing in the PCR reaction; then, during the primer extension, the *Taq* DNA polymerase cleaves out the probe separating the two label molecules, starting the reporter to fluoresce [50, 49]. The fluorescence emission is proportional to the quantity of amplified DNA, since as more PCR products are generated, the more the fluorescence signal increases. In this type of amplification, it is possible to monitor

the production of amplicons in real time, through the measure of fluorescence signal, that generates an amplification curve. These have typically distinct phases: a) lag phase, the initial stage, where there is still no amplification product accumulated to be measured; b) exponential phase, when the reaction components are in abundance and the amplification products are being generated, doubling every cycle; c) linear phase, when the reagents become scarce and the PCR reaction slows down; and d) plateau phase, the final of the reaction. The quantification is based on the fact that the increase in the PCR product are related to the initial quantity of DNA. It is in the exponential phase that the measurements of fluorescence in function of the cycle number are performed, since is when that relationship is more consistent. The value used to do the quantification calculations is the number of cycles needed for the fluorescence to reach a determined threshold – the so-called cycle threshold (C_T), which is detectable over the background noise, in the amplification phase, and is set by the real-time PCR software. The fewer cycles are needed to the fluorescence reach the threshold (i.e., lower the C_T), the higher is the initial quantity of DNA. The obtained curves for casework samples are then compared with standard curves [50].

Besides DNA quantification, available commercial kits provide more information about the genetic evidence due to the specific targeted regions: small autosomal, large autosomal and Y chromosomal portions. The ratio between the first two types of regions delivers an index of degradation (good quality samples amplify small and large fragments in similar proportion, so this index should be low). The quantification of male DNA compared to the autosomal quantification helps to evaluate mixtures with male and female DNA. Moreover, the kits contain an internal PCR control (IPC), which enables to test the presence of inhibitors. Examples of these kits are Quantifiler™ Trio, Investigator Quantiplex and PowerQuant® System.

1.4.4. DNA Amplification

Polymerase Chain Reaction (PCR), firstly described in 1985 by Kary Mullis [27], is one of the most important discovers to molecular science. The capacity to produce a massive quantity of copies of DNA out of small amounts of a specific fragment is an invaluable tool particularly to forensics, where samples are often limited in quantity.

The PCR is based on the natural replication of DNA during the cell cycle, where the DNA content is duplicated. This process was adapted to be executed in vitro, resourcing to an enzyme and to specific DNA fragments to amplify the target sequence.

A PCR reaction contains (a.) a DNA template, from which the copies are obtained, (b.) the enzyme – DNA polymerase, which must be thermostable, to resist to elevated temperature (classically, *Taq* polymerase, a DNA polymerase isolated from a thermophilic bacteria), (c.) primers, fragments of single stranded DNA designed to be complementary to the flanking regions of the sequence of interest; and (d.) deoxynucleotide triphosphates (dNTPs), containing the four bases in similar proportions.

The reaction consists in temperature cycles, provided by thermocyclers, with three stages: (i.) denaturation of the double stranded DNA molecule, (ii.) primers annealing to both strands of the denatured DNA template, (iii.) and synthesis of the new strands by primers extension through addition of dNTPs by the *Taq* polymerase (Figure 1).

This cycle is repeated several times; in each one, every target fragment doubles. Commercial kits containing al the needed components to the reaction are available, significantly simplifying the technique.

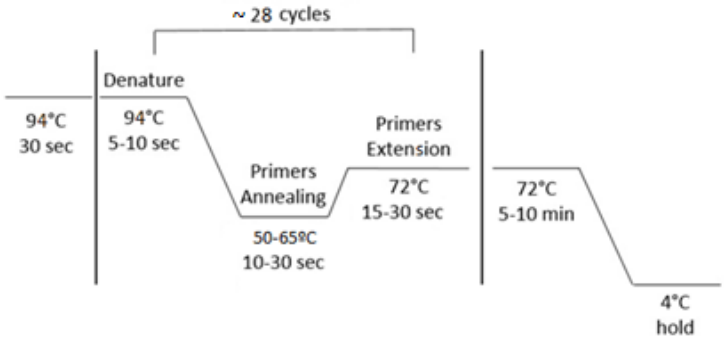


Figure 1. Polymerase Chain Reaction temperature cycles. Adapted from: http://2015.igem.org/Team:Pasteur_Paris/Experiments

Another major benefit of this technique is the possibility of multiplexing, which was developed just a few years later to the PCR description [51]. Multiplex PCR allows the amplification of several target sequences simultaneously at the same reaction, just by adding more sets of primers, directed to the intended regions of the DNA [52]. Current kits employed in forensics consist of STR multiplex kits, i.e., containing multiple pairs of primers directed to the target STRs (Figure 2). These primers have a fluorescent dye bound to one of its ends, which will be used in the next stage.

It is worth to mention that the phases pre- and pos-PCR should be executed in separate locations. Samples from the crime scene and samples from references should also be handled separately in time.

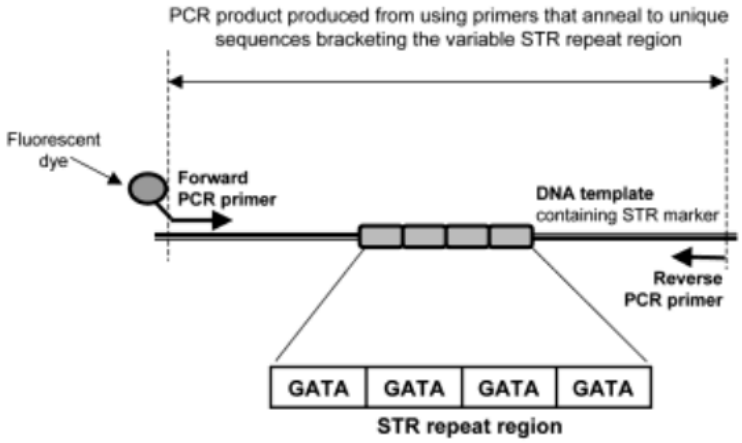


Figure 2. Primers position during DNA amplification process. [55]

1.4.5. STR Separation and Detection

Next to the amplification of the STR markers, they must be separated by length. Formerly, this was achieved by gel electrophoresis and the fragments were detected by silver staining, a laborious and time-consuming method [53]. Currently, the capillary electrophoresis (CE) [54] is the method used and the detection is fluorescent-based. This type of electrophoresis uses electrokinetic injections, where an electric voltage is applied across the capillary – a narrow glass tube, to which the DNA molecules of the samples are drawn according to the electric charge; there, they are separated by length due to a polymer solution on the capillary. A laser light placed close to the end of the capillary detects when a DNA molecule passes by; knowing that smaller fragments move faster across the polymer, the time span from the sample injection to the laser detection correlates to the size of the fragments. For alleles from different loci overlapping in size can be distinguish, the primers used in the amplification are labeled with a fluorescent dye bound; to account for the possible overlaps, in a reaction can be used up to five different dyes. They are excited by the light laser, emitting fluorescence in different regions of the spectra, that is detected by a camera, determining which dye is present, and sending the information to the respective software.

This technique holds a high resolution, allowing for the typing of microvariants too. Besides that, CE has the advantages of being totally automated and using a very small quantity of sample in one analysis (the samples can be reinjected if needed) [55].

1.4.6. STR genotyping

Software programs like GeneMapper® are able to assign the respective alleles to each of the STRs detected.

Along with the samples, an internal size standard and an allelic ladder are injected to the CE. The size standard contains DNA fragments of known size (labeled with a different colored dye); determining the software the size of the alleles from the analyzed samples by comparison with a curve produced by the internal size standard. The allelic ladder contains all the alleles of the loci, previously sequenced; STRs typing is accomplished by comparing the sizes of the alleles of the samples with the alleles of the allelic ladder [55]. Each allele is attributed with a number that represents the number of repeats.

This results in an epg, that contains all the detected alleles organized by marker (that are organized by dye color), in the form of peaks (Figure 3). An epg presents, then, a STR profile, that is, the combination of all the loci genotypes. The peaks are plotted as fluorescent intensity detected versus the time passing through the detector on the capillary on CE (data point). The data point is correlated with the allele size (as mentioned before, smaller sized DNA fragments pass through the detector first, hence having a smaller data point).

The peak height, measured in relative fluorescent units (RFUs), is correlated to the DNA quantity. Bigger the amount of a specific PCR product detected by its fluorescent dye, higher the RFU and, so, the peak height.

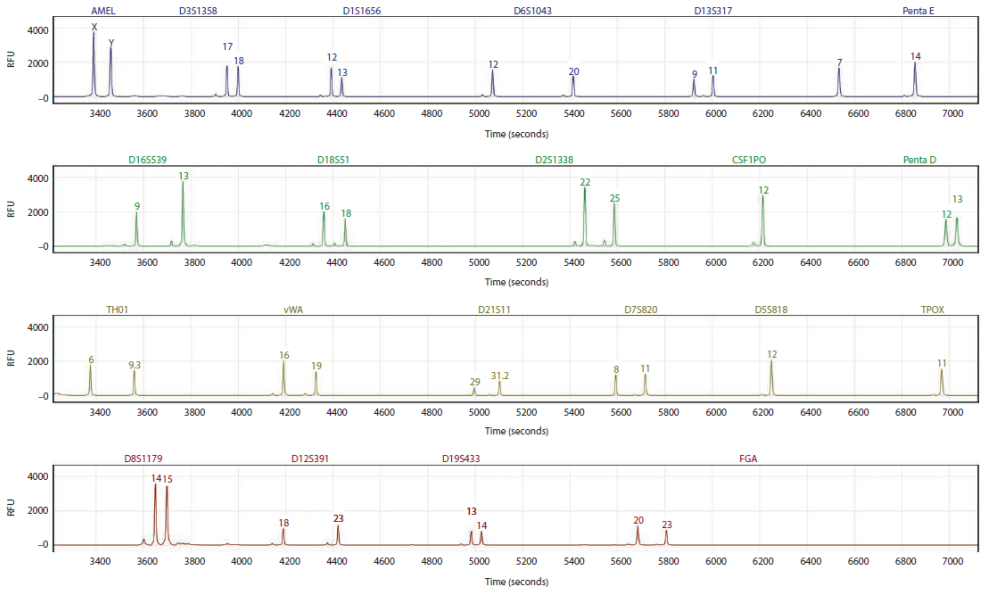


Figure 3. Electropherogram showing a profile with 20 STRs [56].

1.4.7. Profile interpretation

An exhibited peak in an epg is not necessarily an allele, as there are peaks that correspond to artifacts related to the biology of STRs or to the technologies of amplification or detection of PCR products [3, 56].

Despite the automatic evaluation performed by the software, an STR profile must always be reviewed manually by an expert, who verifies if there are artifact peaks incorrectly assigned as alleles, editing if needed. So that the results can be validated, typically, two analysts do the assessment of each profile, separately.

Each laboratory uses a determined threshold limit (e.g.: 50-100 RFUs), which separates analytical from background fluorescence. A too high threshold limit may lead to allele loss; in contrast, with a too low limit background noise and artefactual peaks may be shown [57].

Some laboratories also consider an additional higher threshold – interpretation or stochastic threshold - above which it is reliable that the data is free from stochastic effects and homozygotic peaks can be safely treated as so (as below the stochastic threshold, an apparent homozygous may, in fact, be a heterozygous with a dropped allele) [58].

The training of the analysts is essential, since the effects that can be featured in an epg are several, raising difficulties to its interpretation.

1.4.7.1. Artifacts

The most common artifacts present in a profile are *stutter* peaks (Figure 4) [59]. These peaks are formed during amplification, through a process explained as slippage of the DNA polymerase when extending a new formed strand. It releases from the DNA and the two strands separate as well; when they reattach, a loop is formed if the new strand binds to the template strand one repeat in front of the one supposed. It results in a new fragment that is one repeat (4 bp, for tetranucleotide markers) shorter than what it was supposed to. It usually happens late in the amplification process, and that is why stutter peaks commonly have less than 15% of the correspondent allelic peak [2, 56]. By the position and height, a stutter can be easily identified; however, in an epg with a mixture of DNA donors in which there are minor contributors, some peaks can be very complex to determine if it is a stutter or an allele from one minor contributor.

The so-called stutters generally refer to back stutters (for being placed right before the main peak), but forward stutters are also possible, albeit much less frequent [60]

The probability of stutter occurrence varies according to the STR: shorter core repeats are more prone to this artifact, being this the reason why the markers used in forensics are preferentially tetranucleotides [2].

Split peaks are another biological artifact that may occur (Figure 4) [61]. After copying the DNA template, DNA polymerase adds an adenine to the end of the PCR products. This activity is non-template dependent and happens frequently, being the residue added to the vast majority of the amplified molecules. However, when there is too much DNA or when the polymerase activity is sub-optimal, the enzyme does not add the adenine in all the molecules, resulting some of them one bp longer than the others, to the same allele. One of the split peaks should be assign as "off ladder" by the software. Visually, a peak corresponding to an allele will have the tip split in two (corresponding to the one bp difference).

Regarding to artifacts related to the techniques used in the processing of the samples, one that is common when there is an elevated DNA quantity is *pull-up* (Figure 4). Different dyes used to label different primers can have spectral overlap, which is adjusted by the software, attributing to the fragment the correct color (i.e., in the raw data, a peak is composed of more than one dye color – the correct one and minor ones; after the software correction, it is composed of just one dye color - Figure 5). If the linear range of detection is exceeded due to sample overload, a minor color is "pulled up" to another channel. The result is a minor peak in a different color panel from the major peak from where it was originated, in the same data point. That can help to identify this type of peak, as well as its typical rounded morphology [3; 56].

Residual dye molecules can also be an artifactual peak shown in epgs. These are called *dye blobs* (Figure 4) [62]. They are formed when the fluorescent dyes are not properly attached in the primer synthesis and are released in that phase or come off during the amplification process. The free dye molecule is detected in CE, appearing in the profile as a rounded peak. Dye residues can be removed through a filtration column. Nevertheless, due to their morphology, they can be generally easily identified.

A sharp peak passing through all the color panels is called *spike* and is caused by the detection of crystalized salts in the CE (Figure 4).

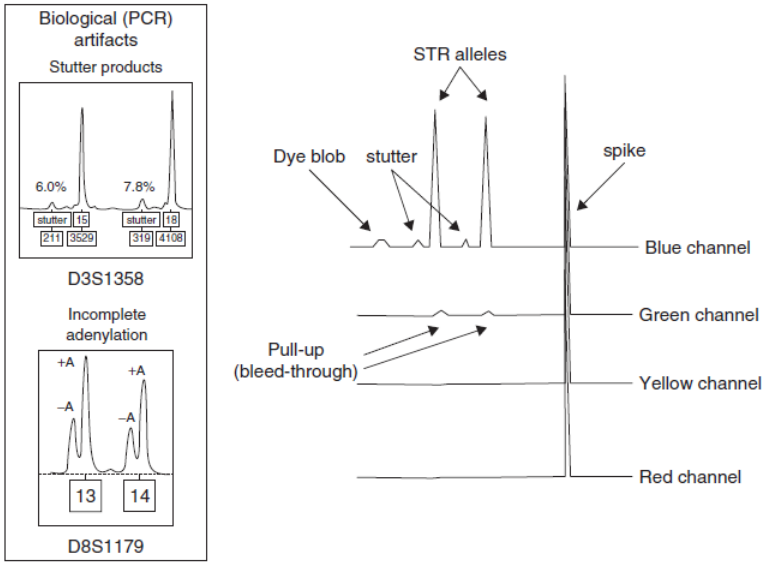


Figure 4. Schematic illustration of several artifacts: stutter peaks, incomplete adenylation or split peaks, dye blob, spike and pull-up. [3]

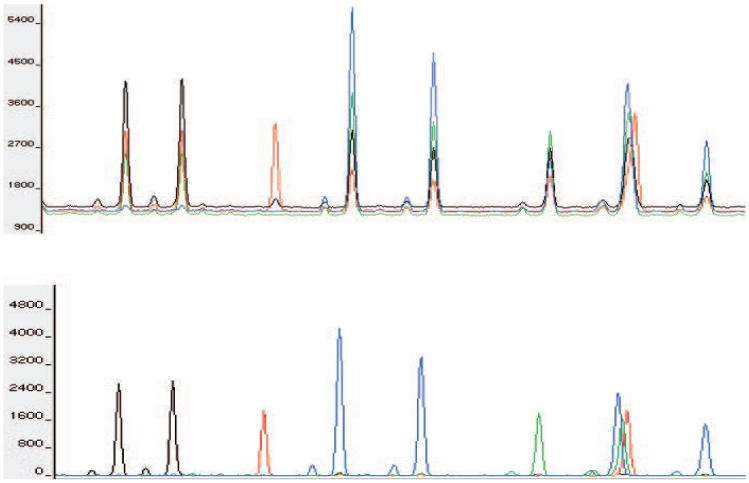


Figure 5. Spectral overlap in raw data (top) and peaks composed of only one dye color after genotyping software correction. [2]

1.4.7.2. Low template DNA

Evidences collected from crime scenes often have a minimal content of biological material. These samples are typically called *Low template DNA* (LT-DNA). A method usually called *Low copy number* (LCN) can be used to process these samples, typically associated with a quantification of less than 200 pg of DNA. It consists in rising the number of PCR cycles to 34 in order to increase the sensibility of the technique [63, 64]. Although it is possible to obtain a profile, great sensibility potentiates the risk of contamination and the incidence of stochastic

effects like elevated heterozygotic imbalance, drop-in or drop-out; and it is also common to verify an increase in stutter peaks [64].

Heterozygote imbalance refers to a situation where the two alleles of an heterozygotic locus have substantial different heights due to the preferential amplification of one of the alleles. Theoretically, the both heterozygotic alleles would have the same height. However, they normally have some variation, due to preferential amplification of one of the alleles, typically the smaller sized one, i.e., the one with less number of repeats. With LCN, this discrepancy increases [65]. If the sample at stake is a mixed profile, the peak imbalance makes the deconvolution of the contributors very challenging, or impossible [66]. The imbalance also can be so pronounced that one of the heterozygotic alleles does has a height below the threshold limit. In this case, it is said that the allele had dropped out.

Allele drop-out is the condition of the presence of a certain allele in the DNA sample that is not displayed in the obtained profile. When drop-out occurs in all the alleles of a locus, it is called *locus drop-out*.

On the opposite, *allele drop-in* can also arise, that is, the presence of a spurious allele that is not from the evidence sample. It is originated from traces of randomly fragmented DNA in the laboratory environment and it should not be amplified in a duplicate reaction [67].

Drop-out and drop-in events create discrepancies between the DNA from the evidence and the reference profile being, therefore, a drawback in profile interpretation.

Nevertheless, the LCN technique is not currently applied very often, since existing kits have a sensibility that allows for the amplification and typing of very low quantities of DNA, without the need to increase the PCR cycles and consequently amplify the risk of the referred effects. However, when the quantified DNA is minimal, the volume of the sample that undergoes through PCR can be increased.

1.4.7.3. Degraded samples

In forensics routine it is usual to recover samples that may have been exposed to the environment for a long time, passing through high temperatures, humidity and microorganisms contamination [12]. In these conditions, the genetic material present in the samples can suffer physical and biochemical degradation, i.e., be fragmented in small portions. If the cleaved sites are located in the polymorphic markers analyzed, its corresponding peaks in the profile will have a lower height than the one that was supposed to (relatively to the true quantity of DNA present in the sample) or will even be undetected. Since larger markers (i.e.,

with higher number of repeat units) are more prone to fragmentation, degraded samples generate a characteristic type of profile (Figure 6), in which the peak heights decrease from the left side of the epg to the right side, showing the amplification success declining as the length of alleles increases.

The interpretation of these profiles is challenging, since they may be partial ones. Though, in some cases the limited information may not be a problem, if the present alleles are rare enough for yield a powerful probative value.

Mixture profiles, however, are much more complex to interpret if the DNA of the contributors is degraded or even if just one contributor DNA is degraded (in this situation, the contributor's relative proportions may vary in different markers).

If the regular STR multiplex amplification does not result in a reliable profile, there are alternatives to analyze degraded samples, like the use of SNP or mini-STRs, since these have a reduced size, are less likely to be fragmented, and so can provide a useful profile [68-70].

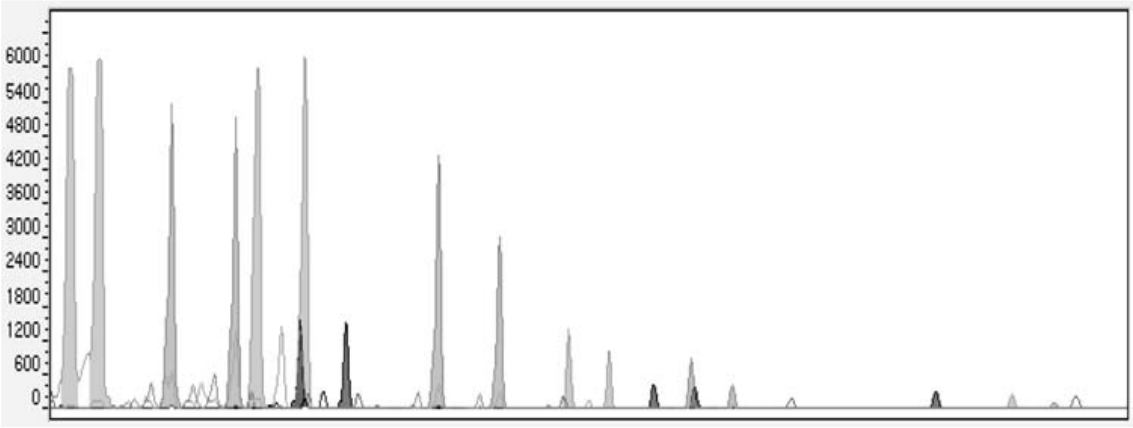


Figure 6. Typical degraded profile. The markers size increase from left to right; the PCR products declines with increased size. Adapted from: [2]

1.4.7.4. Mixed samples

Many collected samples in the context of a crime investigation are composed by biological material from more than one individual, being them involved in the crime (e.g.: DNA of both the victim and the suspect) or as background in the evidence (e.g.: a swab of a steering wheel of a car driven by more than one person). The obtained profile after processing these samples is a mixture of the genetic profiles of its contributors.

The DNA commission of the International Society for Forensic Genetics (ISFG) provided a recommended guideline to the interpretation of mixtures [71].

The first step should be the identification of the presence of a mixture, which is achieved by the presence of more than two alleles per locus, generally in several of them. Notwithstanding it is worth to note that extra alleles may not necessarily correspond to a contributor, but to artifacts or stochastic effects, which is a major barrier in mixtures' interpretation [72]. Also, a large height discrepancy between alleles can be observed, which can be due to allele sharing between the contributors or to different relative quantity proportions between them. Difficulties to detect a mixed profile arise in low quality or partial profiles (due to low quantity DNA and/or degraded) and in the presence of contributors who are genetically related [72].

Next to the mixture detection, the number of contributors must be determined. Note that this is always an estimated parameter, as it is never known how many individuals contributed with their DNA to the evidence. The commonly applied method to estimate the number of contributors is based on qualitative and quantitative information of the epg. The locus showing the maximum number of alleles determines the minimum number of contributors required to explain it - Maximum Allele Count (MAC) - (e.g.: in a certain profile, the locus/loci with more alleles show six, so the minimum number of individuals which can explain it is three, being all heterozygotic). The relative heights of the alleles in the analyzed markers contribute to the estimation of the number of donors too. Additionally, information about the circumstances of the crime may also assist to this stage [73]. It is generally accepted that the determination of the minimum number of contributors is sufficient [74, 71]. Nevertheless, alternative approaches have been suggested, like the estimation of the number of contributors by a maximum likelihood approach [75], and others [76-78].

Since the number of contributors is an estimation (made by the expert, most of the times), it is subjected to error, being possible to under or overestimate it. Bright et al. [79] describe scenarios where these situations are likely to occur. Contributors may be underestimated if: (a.) the DNA of one of them is in so tiny quantity that their presence is unnoticed; (b.) there is a significant allele sharing (e.g.: if the contributors are genetically related) or one donor is masked by other (masking effect) in such form that the peak heights does not allow the inference (c.) if there are multiple low-level donors whom, consequently, suffered drop-out. Concerning the (less common) possibility of overestimate the number of contributors, it generally occurs due to stochastic effects, like a high peak imbalance and/or stutter and drop-in peaks.

Estimate the proportion of each of the components of the mixture is a useful step in its interpretation, knowing that the ratio of DNA template of each contributor in the extracts is maintained through the samples processing and being, therefore, reflected on the height of the peaks. They can be present in similar proportions or it can be possible to distinguish the

minor and major contributors. This determination is preferentially done using markers in which seems that all the contributors are heterozygous, to avoid estimate the ratio based in shared alleles. As the ratio increases, is easier to interpret the major donor; oppositely, the minor donor may be impossible to interpret [72].

Following, the possible genotype combinations should be considered. If the qualitative information brings several genotypic possibilities for a specific set of alleles in a locus, adding the quantitative information and the determinations done in the previous step, helps to delimit the possibilities (if there is a clear minor and major contributor) [72].

Only after these determinations, the reference sample from a putative contributor must be considered and compared with the mixture profile, to avoid the possibility of a biased interpretation. If the profile of a person of interest (POI), matches the determined genotypes, it cannot be excluded from having contributed to the mixture [71].

1.4.8. Quantification of the weight of the DNA evidence

When a DNA profile from a POI does not match a good quality profile from an evidence recovered from the crime scene, it is reasonable to say that the suspect is excluded from having contributed with his/her DNA to the sample. In contrast, when a match is observed, it cannot be excluded that the POI contributed with his/her DNA to the sample. However, it cannot be categorically stated that the individual had contributed to the sample, since there is the possibility that the evidence does not contain DNA from the POI, but the profiles coincide by chance, i.e., it is a random match. Therefore, the weight of the evidence must be quantified. Reversely to the classical fields of forensics that rely on the principle of discernable uniqueness, forensic genetics does not individualize, but calculates expected frequencies for types of observations [80].

The assessment of the probative value relies on the assumption that every DNA profile occurs with a certain frequency in the considered population. An evidence profile containing rare alleles (i.e., with low frequency), delivers a more powerful evidence.

The recommended method by the DNA Commission of the ISFG [71] and widely accepted by the scientific community for the quantification of the proof is the calculation of a Likelihood Ratio (LR) [81], which opposes two alternative and mutually exclusive hypotheses on the origin of the genetic material of the evidence. In the context of an identity test and a mixture evidence, the hypotheses generally state that a certain reference profile is a

contributor of the mixture (H1) and that a certain reference profile is neither a contributor of the mixture, nor genetically related to a contributor (H2).

The probabilities of observing the evidence (*E*) under these two hypotheses are calculated using the frequencies of the alleles of the evidence and are then compared:

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

According to Bayes' Theorem, posterior odds are calculated by multiplying prior odds (which considers other type of data) with the LR computed based on the genetic evidence [82]:

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

The *a priori* probabilities of each of the hypotheses – P(H1) and P(H2) – are, generally, considered to be the same. Therefore, the posterior odds are equivalent to the LR computed by the forensic genetics' expert.

A LR greater than one favors the H1, and H2 is favored by an LR inferior to one (assuming *a priori* odds equally likely). The obtained result represents how many times it is more likely to observe the evidence assuming one hypothesis, than assuming the other. The interpretation of the obtained LR value is not objective, in terms of include or exclude a POI; instead, it provides different levels of support to the defined hypotheses.

It is worth mention that there is a common misconception of the LR, which is stating that the result represents a probability of identity or how many more times one hypothesis is more likely than the other. This falls within the fallacy of the transposed conditional or the prosecutor's fallacy [83], i.e., the likelihood of the hypothesis given the evidence, P(*H*|*E*).

In contrast to other approaches to assess the probative value, LR can account stochastic effects that may occur during samples processing and is, consequently, the generally considered most suitable method to what is the forensics reality.

Another method, currently in disuse, is a simple frequentist approach to evaluate the significance of the evidence, like the Random Man Not Excluded (RMNE). It considers how often a random individual from the population would be excluded as a contributor of the observed evidence. Although this method has the advantages of being easier to explain on court and not require estimates of the number of contributors, it entails a binary vision of alleles, as it does not consider stochastic effects and neglect information that could be used in the statistical assessment, since it does not depend on the genotype of the POI at stake, hence being a less powerful method compared to LR. RMNE relies on an unrealistic simplistic view of DNA evidence [84, 85].

1.4.8.1. Interpretation models

Several interpretation models are based on the calculation of the statistical weight by the LR method. The simplistic binary models assign a value of zero or one (non-match or match) to the evidence, depending on the observed data. This was achieved considering genotypes as possible or impossible, initially just based on the presence/absence of alleles and, afterwards, based on heterozygote balance and mixture proportion [86].

With the introduction of software-based [87] probabilistic methods, the probabilities assigned for the assumed hypotheses could have any value from zero to one, solving the issue of binary models of not dealing with a locus showing a non-concordance [84, 86]. Accordingly, semi-continuous or qualitative models introduced the assignment of events of drop-in and drop-out to observed and missing alleles, respectively (for this reason these models are also called *drop-models*). It includes on the probabilistic calculation, the probability of a dropped allele – Pr(*D*) – and allelic contamination – Pr(*C*) [87]. An example of a semi-continuous interpretation software is LRmix Studio [74; 89].

Continuous or quantitative models added the advantage of considering quantitative information, such as the height of the peaks (using it as continuous variables), into the assessment of the possible genotype combinations. This approach is also capable of modeling stutter peaks. Due to the consideration of stochastic effects and artifacts, these models are able to handle any non-concordance that may occur [90]. Euroformix is one of the software programs using quantitative models currently available [91].

Although the evaluation of a probative value through the computation of the LR using the quantitative model can be complex to explain in court, that should not overlap to the fact that it is the method which does the wider use of the available information provided within an epg and, consequently, the most appropriate.

1.4.8.2. Parameters influencing the quantification of the proof

The resulting LRs obtained through the currently used approaches (qualitative and quantitative) depend not only on the frequency of the alleles in the population, but are also influenced by other parameters, such as:

1.4.8.2.1. Number of contributors

As already mentioned (see 1.4.7.4), when dealing with a genetic evidence that consists on a mixed profile, the number of donors must be estimated (as the correct number is never known). However, this is a challenging task due to effects such as allele sharing between donors and stochastic events. Attention to this topic and its implication in LR computations has been devoted through empirical analysis [92, 93, 73].

1.4.8.2.2. Co-ancestry coefficient (F_{ST})

The calculation of a genotype frequency relies on the Hardy-Weinberg model, which entails some rules to be met in the population: be infinitely large, have random mating, free from effects of migration, free of natural selection, and no occurrence of mutations. Most populations do not meet these criteria, being sub-structured and its allele frequencies (and consequently profiles) varying between subpopulations. Within a subpopulation, there is a higher level of relatedness (relatively to the whole population) and, so, there is a higher probability that a shared allele between two individuals to be identical by descendent (IBD), that is, it descends from a common ancestor. Hence, if this is not taken into account, it leads to a wrong estimation of the profile frequencies [94]. Consequently, it is required to correct the statistical evaluation through the application of what it is called theta (θ) or F_{ST} in the calculations of profile frequencies [95]. This parameter is an empirical determined measure of population substructure. However, most laboratories with forensic casework routine, considers assigned values for similar populations, also based on previous recommendations [94].

1.4.8.2.3. Drop-in

As stated before (see 1.4.7.2), it is possible that alleles non-related to the crime appear in the epg. Existing qualitative and quantitative interpretation software are able to handle with the possibility of drop-in occurrence when computing a LR. For modelling drop-in occurrence is required to introduce the respective probability, as well as a parameter related to the height of drop-in peaks in the case of quantitative models, the so-called lambda, λ. Higher is the λ value, higher is the sensitivity for peak heights, i.e., peaks little differentiated regarding to heights can have very different probabilities of being considered as a drop-in (given that the drop-in probability always decreases with height increase) [96]. Ideally, a drop-in probability should be

estimated to each profile using negative controls; however, it is common practice to use a default value in forensics routine [97].

Depending on the assigned drop-in probability, the observed alleles of an evidence are attributed with a certain weight: as the drop-in probability decreases, the chance that an observed peak on an epg belongs to a contributor increases.

1.4.8.2.4. Threshold limit (T)

A threshold limit is a value (measured in RFUs) beyond which peaks are considered an allele, separating them from baseline noise. A large threshold limit ensures the exclusion of noise signals from the genetic profile and, thus, that they are not assigned as alleles; however, it may also lead to information loss, since smaller alleles may not be detected. On the other side, a low threshold reduces the chance of data loss, but increases the possibility of noise peaks on the profile. With this in mind, it must be found a balanced threshold limit that minimizes the effects of drop-in and drop-out [57].

2. Aims

Under the framework of identity testing, the main aim of this work is to compare computed Likelihood Ratios (LRs) obtained through different statistical models, mostly using real casework mixtures and references.

For this, we compare LR results:

(a.) Obtained through two software programs, one based on a qualitative model and other on a quantitative one;

(b.) Considering mixtures with different estimated number of contributors;

(c.) Varying the estimated number of contributors of each mixture for both real and simulated (1st degree relatives of the first) references;

(d.) Varying several parameters related to populational sub-structure and analytical factors, that are introduced in the software by the user for the casework references;

3. Methods

3.1. Sampling

3.1.1. Real casework samples

Although recognizing that mocked samples can be used to study the performance of software programs, in this work we mainly considered real criminal casework samples since these have a multifactorial complexity associated that are difficult to replicate. Thus, we sought to observe the behavior of the informatics programs using this type of samples. In this regard, the samples chosen to be part of this work were evidences selected from former cases of the Laboratório de Polícia Científica da Polícia Judiciária (LPC-PJ), with the criteria of being mixtures and have at least one reference (single profile) associated.

The reference STR profiles were obtained previously by the LPC-PJ through sampling of one individual that was considered to be a possible donor of the corresponding mixture, based on the police investigation. Consequently, in the respective caseworks, they were analyzed as POI and it was concluded that they could not be excluded to be a contributor of the mixture. Some of the references used in this work have a different background, as they do not were sampled from an individual, but are single profiles obtained in the same case that the respective mixture, that were also not excluded to belong to a contributor of the mixture.

The samples were previously processed in the context of the respective casework, through extraction in Automate Express™ Forensic DNA Extraction System, using the PrepFiler Express BTA™ Forensic DNA Extraction kit; quantification in the equipment 7500 Real-Time PCR System, using the Quantifiler™ Trio DNA Quantification kit; amplification in thermal cycler GeneAmp® PCR System 9700 using GlobalFiler™ PCR Amplification kit; and, finally, PCR products detection and separation by capillary electrophoresis in 3500 Genetic Analyzer. The work under the scope of this thesis initiated with the search for the required genetic profiles, which were found in report format (in tables).

3.1.2. Simulated samples

In this work we also analyzed the behavior of the computer programs when a close relative of the reference was considered as the POI and the number of contributors varied. To do so, profiles of one full-sibling and one parent were simulated for each reference through algorithms computed in software R. These profile simulations were conditioned to the alleles

of the reference profile. To simulate a profile of a parent of the reference, to each marker was attributed an allele of the reference on that marker, each of the two alleles with 50% of probability. The other allele of the parent to that marker was generated considering the cumulative frequency of the alleles in the population (to each allele corresponds the sum of the frequencies of the previous alleles). It is worth to note that this approach reflects the frequency of the alleles in the population as more frequent alleles have a wider interval associated. Random numbers between 0 and 1 were generated and the attributed allele is the one corresponding to the interval where the random number situates.

To simulate a full-sibling of the reference, the genotypes of their parents must be simulated first. To each marker, one parent was attributed with one allele of the reference, and the other parent with the other allele. The lacking allele in each parent was generated by the same method described above. Next, each parental allele was considered to be transmitted with 50% of probability to the full-sibling that is being simulated.

3.2. Profile Interpretation

The fsa files of the casework mixtures and corresponding reference profiles considered, were recovered so that they could be analyzed through the typing software GeneMapper® ID-X - with a threshold limit of 100 RFUs, in order to attribute the present alleles and obtain the electropherograms. In this stage, we decided about the peaks presented in the electropherogram, eliminating those that we considered that were not allelic but an artifact or a stochastic effect (see Chapters 4.7.1 and 4.7.2).

Afterwards, we estimated the number of contributors to each mixture by observation of the electropherogram through allele count per marker and their relative heights. Profiles with four or more apparent donors were discarded of this study, as they can be too complex to interpret.

As so, the final selected sample for this study was composed by 79 mixtures with two contributors estimated, and by the same number of mixtures with three contributors estimated. For each one of these 158 mixtures we considered one real casework reference sample, and one parent and one full-sibling simulated from the previous real profile.

3.3. Statistical Analyses

For each pair mixture/reference (real or simulated), a weight of evidence was calculated in the form of the LR, assuming as hypothesis: “the POI is a contributor of the

mixture" (in the numerator) and "the POI is genetically unrelated to any contributor of the mixture" in the denominator (see 1.4.8). Note that when using the simulated references, we are not following the assumptions of the software and therefore the results will be biased. Nevertheless, with this approach we intended to simulate the case where, unknowingly, a relative of a reference compatible with a profile of the mixture is analyzed under the assumptions stated above (which are those generally considered).

LRs were calculated through two computer programs of interpretation of forensic samples: one using a quantitative model – Euroformix version 1.9.3; the other using a qualitative model - LRmix Studio version 2.1.3 (see 1.4.8.1).

The weighing of the probative value depends on the allelic frequencies of the population and, in this work, we considered the database of the National Institute of Standards and Technology (NIST) concerning the Caucasian population (see Appendix I).

The number of contributors (see 1.4.8.3) is a setting that must to be introduced by the user in the software before each analysis. This parameter is not known, being only possible an estimation by the expert, depending on his/her evaluation of the electropherogram, in each case. Given that the common bad quality and low quantity of DNA present in forensic evidences this can be a complex assessment, as it is possible that this parameter can be incorrectly estimated by being under or overestimated. Hence, these circumstances were experienced in this work, by computing a LR to a reference profile inputting a number of contributors that was below and above the number estimated. Precisely, for real pairs mixture/reference, mixtures assumed as having two contributors were also analyzed considering three; and mixtures assumed as having three were also analyzed assuming two and four contributors. On the other hand, and due to time constraints, for simulated reference profiles only mixtures with three donors estimated were analyzed with under- and overestimation.

In each of the computer programs, specific parameters can be introduced by the user. For those chosen to be tested in this work, we established some values, designated hereafter as "default values", which we considered reasonable for urban populations, taking into account the default values on the software, as well as values presented in the literature. The LRs were computed to all real and simulated references with these values. These obtained LRs are those used as benchmark for comparison with the LRs resulting from the variation of number of contributors and of other parameters, described next. Setting the parameters to the same default value on the two computer programs, allowed us to compare the results obtained through the both.

Then, we determined variations of the default values (under and above these), for each parameter, as described below in sections 2.3.1 to 2.3.3 (see also Table 1). Using those varied values, LRs were calculated for real casework references.

The LRs were computed varying the parameters each at a time, so it was possible to perceive the effect of the variation of each parameter separately. The impact on the probative value of each variation was measured by subtracting the LRs in 10-log scale (which is equal to the $\log_{10}$ of the ratio of the two LRs at stake).

Note that all the LR variations were compared not only intra-, but also inter-computer programs.

In total, 3950 LR computations were performed.

3.3.1. Co-ancestry coefficient

For F_{ST} (see 1.4.8.4), the defined default value was 0.01. This value corresponds to the default in LRmix Studio, and is sustained by studies with similar populations where this value was used, and on what was recommended as a value for a broad geographic group [97-99, 79], being however referred as a too conservative value for urban populations in some studies [100, 101]. Beyond the default value 0.01, the varied values of F_{ST} tested were: (a.) 0, representing a situation where the effect of subpopulation is ignored; (b.) 0.03, a value that already was recommended for broad geographic groups [99] but meanwhile was considered extremely conservative for nowadays populations [102]; and (c.) 0.015, an intermediate value to verify a possible trend on LR increase or decrease.

3.3.2. Drop-in

The probability of drop-in (see 1.4.8.5) was set to 0.05 as default, a value considered as reasonable in similar studies [97, 73], coinciding with the pre-set value in LRmix Studio. The variations made in this parameter were: (a.) 0, meaning that alleles not attributed to a contributor cannot be considered as a drop-in peak; (b.) 0.1, a high probability of unexplained alleles being a drop-in. Still related to drop-in peaks, but now regarding to the impact of the height of them, the so-called parameter lambda λ (only included in Euroformix), was also tested with a default value of 0.01 (corresponding to the default value on that software), and a varied one of 0.05 (the λ cannot be equal to zero).

3.3.3. Threshold limit

Likewise, the threshold limit (see 1.4.8.6) is a parameter only allowed to be introduced by the user in the quantitative software Euroformix; so only in this program it was varied and tested. Its default value is 100 and the varied value was 150. With this latter setting, some higher peaks were not considered in the LR computation.

Table 1. Default and varied values inputted on the software for each parameter.

	Euroformix	***LRmix Studio***	***Default***	***Variations***
F_{ST}	0	0.01	0.01	0
				0.015
				0.03
Drop-in	0	0.05	0.05	0
				0.1
λ	0.01	-	0.01	0.05
T	50	-	100	150

3. Results and Discussion

3.1. Weighing the evidence with real casework references

3.1.1. With the estimated number of contributors

3.1.1.1. Default parameters

Figure 7 shows the results of the analyses computed considering a profile of the POI, weighing the likelihoods of the observations assuming that the POI contributed to the mixture and assuming that the POI is genetically unrelated with any contributor of the mixture. These LRs were obtained through: (a.) LRmix Studio (qualitative model), and (b.) Euroformix (quantitative model). In this experiment, the analyses were computed for each case assuming the estimated number of contributors through the observation of the epg, namely two (n=79) and three (n=79) contributors. All the parameters which are needed to be introduced by the user (detection threshold, co-ancestry coefficient, drop-in properties) were set to what we considered as default values – see Table 1.

As can be seen in Figure 7, LRs calculated through Euroformix were generally greater than those computed with LRmix Studio for both types of mixtures (with two and three estimated contributors). In fact, only three cases out of 158 resulted in a higher LR obtained by LRmix Studio; however, these specific values were not much distant from those obtained by Euroformix (mean difference of less than one).

Since Euroformix takes quantitative information related with the peak heights into account (besides the allelic frequencies), it quantifies the likelihood of the different genotypic combinations based on the quantity of DNA. Indeed, the software attributes more weight to markers where the alleles of the POI match a possible genotype set that the software assumes as more likely, relatively to others, due to the peak heights. Consider for example a mixture where, for a specific marker, exactly the four alleles 12, 14, 16, 17 were undoubtedly identified. Alleles 12 and 16 have similar peak heights, as well as alleles 14 and 17, notwithstanding the uneven DNA quantity between the two pairs of alleles. Assuming two contributors, this scenario provides three possible pairwise genotypic configurations: 12-14 and 16-17; 12-16 and 14-17; or 12-17 and 14-16. However, the pairs of alleles with similar height are the most likely genotypic configurations; in this example, 12-16 and 14-17. If the POI has one of these genotypes on this marker, the quantitative model will attribute it a higher weight (than if she/he has one of the other genotypes). On the other hand, in the qualitative model,this information is not considered in the analysis. Therefore, the overall LR calculated

for a true contributor of a mixture is expected to be superior when computed in a quantitative model, comparing with a qualitative one. This explains some large differences found between the LRs computed for the same sample (mixture/reference pair), through different programs.

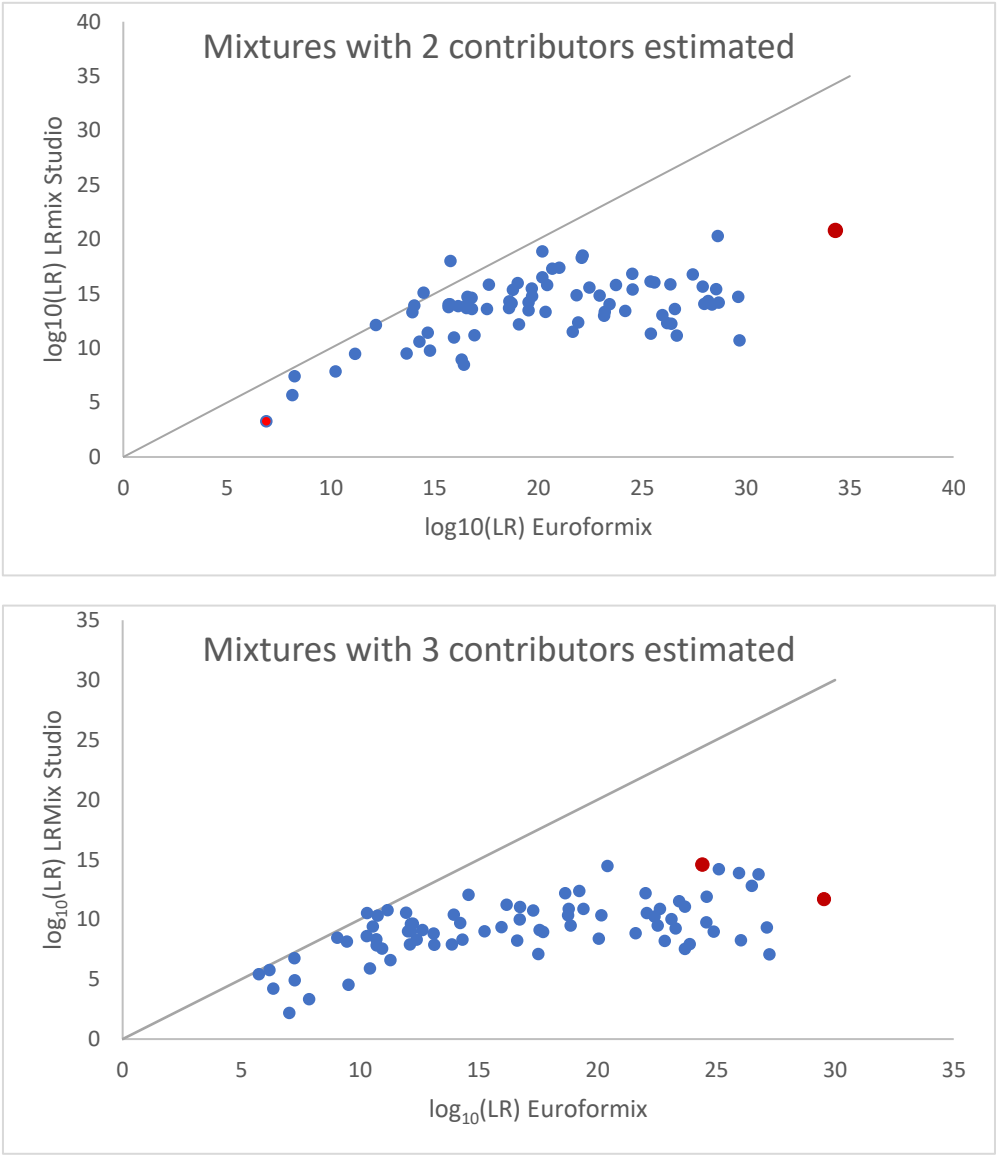


Figure 7. Plots showing the obtained LRs (in log_{10} scale) through Euroformix and LRmix Studio, regarding the same samples. The upper plot is regarding mixtures with two estimated contributors; the lower plot is regarding mixtures with three estimated contributors.
The line represents $log_{10}(LR)EFM=log_{10}(LR)LRmixStudio$. The red dots represent the maximum LRs.

Table 2 shows the differences between the LRs calculated by the two considered software in a more detailed way. The $log_{10}(LR)$ from one software were subtracted to the corresponding $log_{10}(LR)$ from the other (which is equivalent to the log of the ratio of the two LRs). For mixtures of two estimated contributors, it was observed that 25% of the calculated LRs through the two software, were separated by more than ten units on the log_{10} scale, being

the largest difference of about 18. Regarding mixtures of three estimated contributors, 32% of the LRs differed by more than ten units on the same scale; here, the largest variation was of about 20.

Concerning the mixtures assumed to have two contributors, the maximum value of $\log_{10}$(LR) obtained through Euroformix was 34.30; in qualitative software LRmix Studio this value was 20.83, both obtained for the same sample (pair mixture/reference). While in mixtures with three contributors assumed, the maximum values obtained for $\log_{10}$(LR) were of 29.54 in Euroformix, and of 14.60 in LRmix, this time corresponding to different samples.

All the $\log_{10}$(LR) are presented in Appendix II.

Table 2. Distribution of the differences between the LRs ($\log_{10}$ scale) obtained by Euroformix and LRmix Studio for C estimated contributors, and the maximum, mean and median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

$x=\log_{10}(LR_H/LR_L)$	C=2	C=3
$0 < x < 2$	15%	14%
$2 < x < 4$	24%	15%
$4 < x < 6$	14%	18%
$6 < x < 8$	11%	10%
$8 < x < 10$	10%	11%
$10 < x$	25%	32%
Max	18.97	20.17
Mean	6.70	7.53
Median	5.00	6.47

3.1.1.2. Varying parameters

3.1.1.2.1. Probability of drop-in

The probability of occurrence of drop-in was varied to lower and higher values relatively to the considered default value - 0.05, specifically to 0 and 0.1 – see Table 1.

Globally, these variations did not have much impact on the calculated LRs. As shown in Table 3, the great majority of the tests performed with varied drop-in values produced LRs with a difference of within one unit on the $\log_{10}$ scale, relatively to the LRs calculated with the default value. In fact, only in the variation to a null value in Euroformix were obtained LRs that differed in more than one unit on the $\log_{10}$ scale.

In quantitative software Euroformix, the larger differences between LRs were all decreases and were obtained in analyses with the probability of drop-in set null, in mixtures that have at least one marker where, although the number of peaks does not exceed the maximum number that

is possible to belong to the number of contributors defined, the model infers that are more alleles than the ones that are possible, based on their relative heights (Figure 8).

In both software, when the number of peaks surpass the maximum that is possible to the defined number of contributors (or is the maximum possible but the reference is homozygous), the model cannot explain the data if the probability of drop-in is null, i.e. there are alleles that are not explained by the contributors nor drop-in, and it is not calculated a LR.

Table 3. Distribution of the differences between LRs (log_{10} scale) obtained with the varied values of probability of drop-in (0 and 0.1), comparing to those obtained with the default value (0.05), for C estimated contributors; and the maximum, mean and median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

	C = 2				***C = 3***			
	Euroformix		***LRMix Studio***		***Euroformix***		***LRMix Studio***	
$x=log_{10}(LR_H/LR_L)$	***0***	***0.1***	***0***	***0.1***	***0***	***0.1***	***0***	***0.1***
0 < x < 1	90%	100%	100%	100%	91%	100%	100%	100%
1 < x < 2	2%	-	-	-	4%	-	-	-
2 < x < 3	3%	-	-	-	1%	-	-	-
3 < x < 4	2%	-	-	-	0%	-	-	-
4 < x	3%	-	-	-	4%	-	-	-
Max	5.89	0.96	0.08	0.91	6.71	0.76	0.07	0.31
Mean	0.42	0.10	0.03	0.08	0.37	0.09	0.02	0.03
Median	0.02	0.01	0.03	0.04	0.06	0.05	0.01	0.02

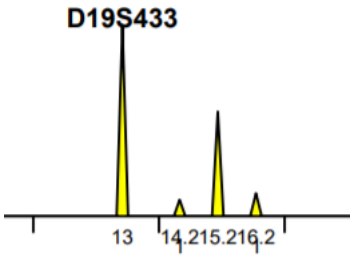


Figure 8. Representation of a marker with four alleles from a mixture with two estimated contributors and uneven relative peak heights, according to the number of contributors defined. This exemplifies a situation where by nulling the drop-in probability, alleles remain unexplained by the contributors and settings defined (hence lowering the LR).

The parameter λ, which influences the probability of drop-in depending on the height of peaks was also varied on Euroformix (the only software where this parameter was considered). Such as in the variation of the probability of drop-in, the LR was not greatly affected by the modification of the *λ* value (Figure 9).

Table 4 shows the distribution of the differences between the LR when the analyses were made with the default value of λ (0.01) and the LR when λ was changed to 0.05 – see Table 1. The differences were within one unit on the $\log_{10}$ scale in most part of the analysis, for both two contributors' mixtures and three contributors' mixtures. Nevertheless, a few larger differences were found when varying λ in mixtures of two estimated contributors, precisely decreasing of the LR. Probably because unattributed peaks that were no longer possible to be explained by drop-in.

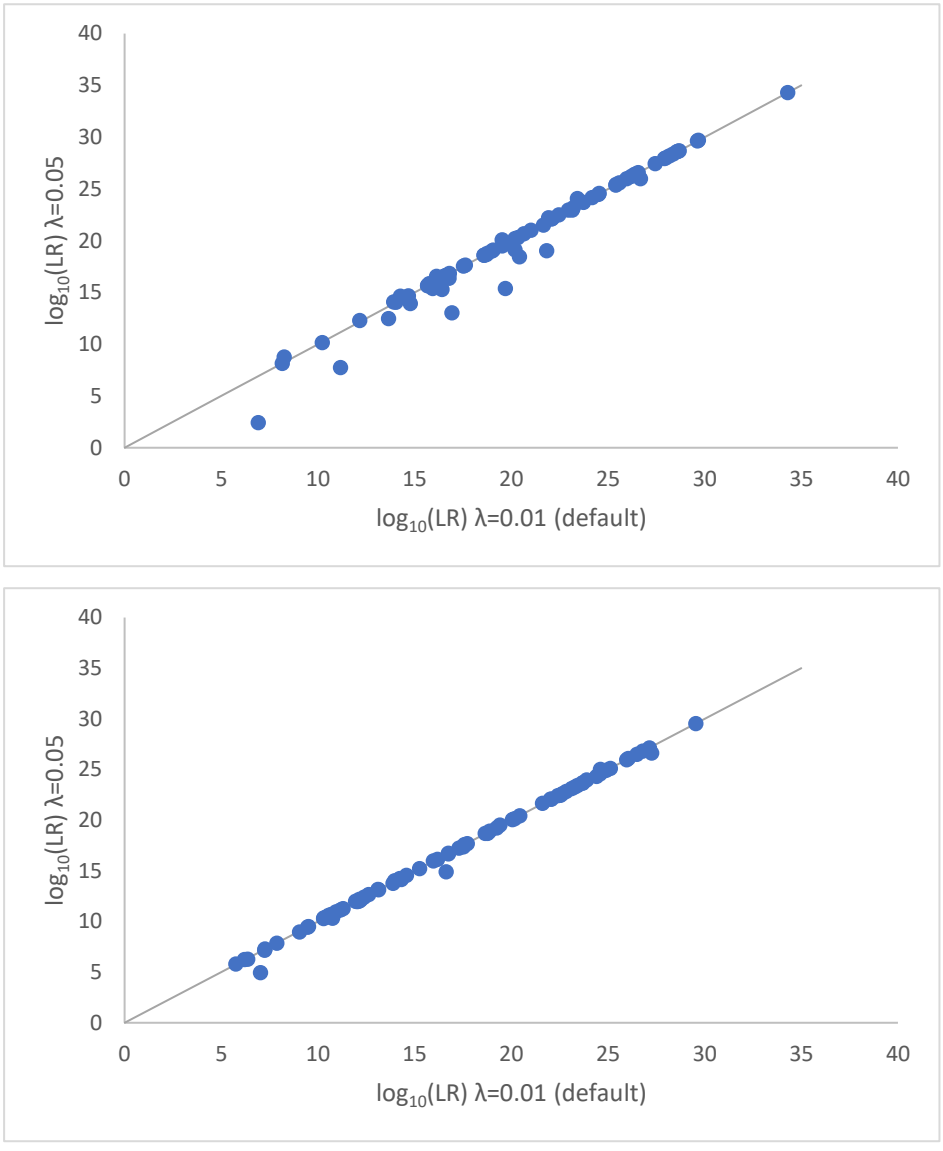


Figure 9. Plots showing the obtained $\log_{10}$(LR) for λ=0.01 (default value) and for λ=0.05, on Euroformix, for mixtures with two (upper plot) and three (lower plot) estimated contributors.

Table 4. Distribution of the differences between LRs (log_{10} scale) obtained with the varied value of λ (0.05), comparing to those obtained with the default value (0.01), for C estimated contributors); and the maximum, mean and median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

$x=log_{10}(LR_H/LR_L)$	C = 2	C = 3
$0 < x < 1$	89%	97%
$1 < x < 2$	5%	1%
$2 < x < 3$	1%	1%
$3 < x < 4$	3%	0%
$4 < x$	3%	0%
Max	4.49	2.09
Mean	0.39	0.10
Median	0.02	0.03

3.1.1.2.2. Co-ancestry coefficient (F_{ST})

Through comparison of the obtained LRs when the default value of F_{ST} (0.01) was varied to 0, 0.015 and 0.03 (see Table 1) it become evident (as expected) a linear tendency: higher values of F_{ST} lead to lower values of LR (Figure 10 and Appendix III). This was verified in all the cases.

This was the expected result, since with a higher F_{ST}, a match between an allele of the reference and the mixture has a higher probability of being an allele shared by descent and so, the attributed LR must be lower.

The amplitudes of the LR differences corresponding to the comparison of the results considering the default value (0.01) and each varied value are presented in Tables 5 and 6.

It was also noted that the instances where the F_{ST} variation had a larger impact were in the cases of mixture samples with several alleles with low frequencies in the population (on both software). This is coinciding with previous studies [99]. A rare allele is a feature that sustains and gives more weight to each of the two situations represented by an increase/decrease of the F_{ST}. Considering a situation where there is higher probability of identity by descent, if a matching allele is rare, it sustains the possibility of IBD, lowering the LR significantly (comparing to an identical situation with a frequent allele, where the impact of IBD is expected to be lower). On the other hand, with a reduced possibility of IBD alleles, a match between a rare allele of the reference and the mixture, gives more weight to the hypothesis of identity (compared to the same situation with an allele with higher frequency).

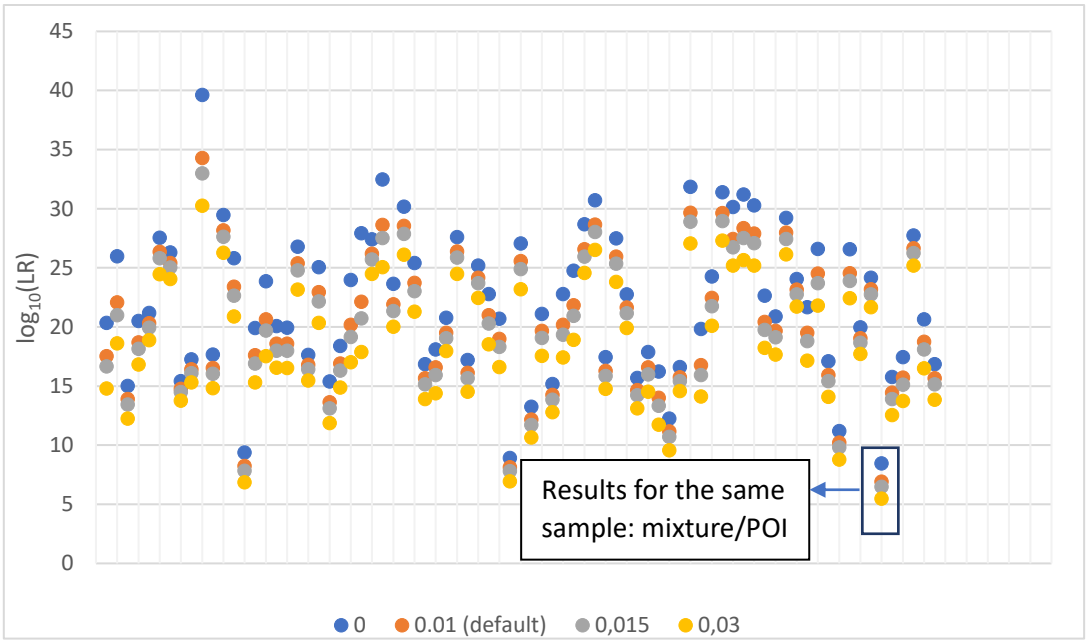


Figure 10. Plot showing the LRs obtained by Euroformix when the F_{ST} correction is varied in mixtures of two person estimated. Each vertical group of dots represents the LRs obtained for the same sample when F_{ST}=0 (blue dots), 0.01(default value; orange dots), 0.015 (grey dots) and 0.03 (yellow dots). Each set of four dots with the same x corresponds to the results obtained for a single sample (mixture/reference). The same exact trend was observed in both software and in both type of mixture (Appendix III).

Table 5. Distribution of the differences between LRs (log_{10} scale) obtained with the varied values of probability of F_{ST} (0, 0.015 and 0.03), comparing to those obtained with the default value (0.01), for two estimated contributors; and the maximum, mean and median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

	Mixtures of two estimated contributors					
	Euroformix			***LRMix Studio***		
$x=log_{10}(LR_H/LR_L)$	***0***	***0.015***	***0.03***	***0***	***0.015***	***0.03***
0 < x < 1	15%	94%	0%	20%	18%	0%
1 < x < 2	54%	6%	49%	54%	76%	71%
2 < x < 3	22%	0%	43%	16%	6%	27%
3 < x < 4	6%	0%	5%	6%	0%	3%
4 < x	3%	0%	3%	3%	0%	0%
Max	5.81	1.39	4.24	5.32	2.45	3.64
Mean	1.76	0.61	2.08	1.65	1.32	1.88
Median	1.49	0.58	2.01	1.33	1.30	1.82

Table 6. Distribution of the differences between LRs (log_{10} scale) obtained with the varied values of probability of F_{ST} (0, 0.015 and 0.03), comparing to those obtained with the default value (0.01), for three estimated contributors; and the maximum, mean and median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

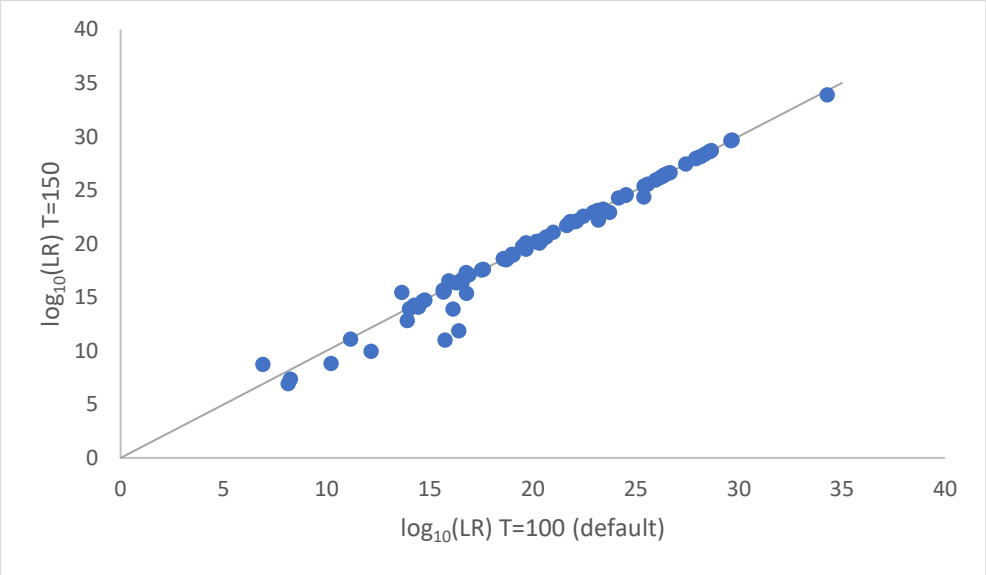
	Mixtures of three estimated contributors					
	Euroformix			***LRMix Studio***		
$x=log_{10}(LR_H/LR_L)$	***0***	***0.015***	***0.03***	***0***	***0.015***	***0.03***
0 < x < 1	24%	99%	6%	38%	100%	6%
1 < x < 2	57%	1%	52%	46%	0%	78%
2 < x < 3	15%	0%	38%	14%	0%	15%
3 < x < 4	4%	0%	4%	3%	0%	0%
4 < x	0%	0%	0%	0%	0%	0%
Max	3.86	1.01	3.25	3.93	0.94	2.87
Mean	1.51	0.55	1.88	1.37	0.48	1.60
Median	1.45	0.55	1.86	1.28	0.48	1.60

3.1.1.2.3. Threshold limit (T)

Figure 11 shows the impact on the LR of varying the threshold limit of peak detection, which was tested considering the default value 100 and the varied value 150 (see Table 1).

As seen on Table 7, the majority of the differences on the computed LRs for the two mentioned values were within one unit on the log_{10} scale. However, a small percentage of samples yield considerable differences – until about four units on the same scale.

All these larger differences consisted of LR decreases and correspond to cases where the extension of the limit of detection eliminated some alleles that matched with the reference.



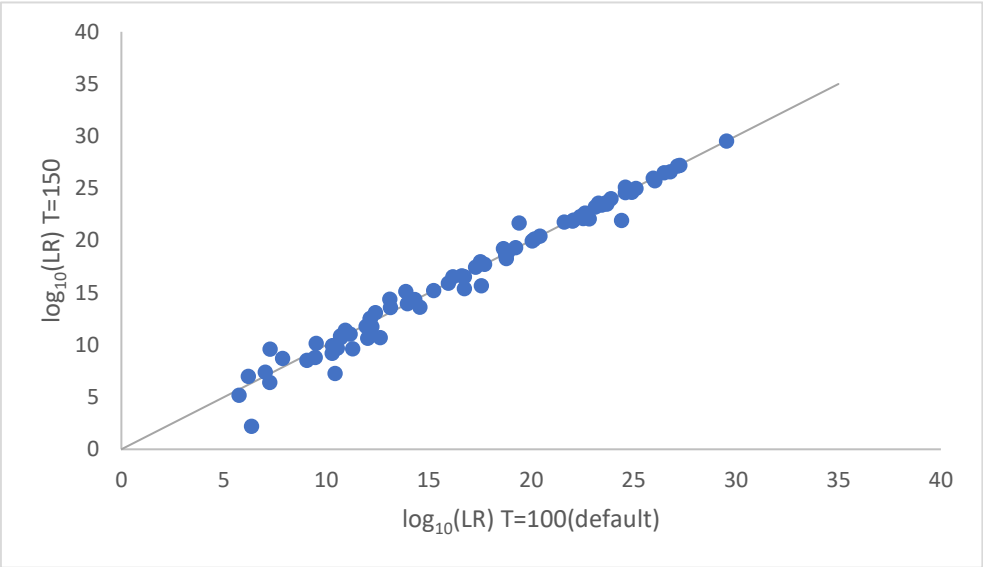


Figure 11. Plots showing the obtained log_{10}(LR) for T=100 (default value) and for T=150, on Euroformix, for mixtures with two (upper plot) and three (lower plot) estimated contributors.

Table 7. Distribution of the differences between LRs (log_{10} scale) obtained with the varied value of threshold limit (150), comparing to those obtained with the default value (100), for C estimated contributors; and the maximum, mean and median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

$x=log_{10}(LR_H/LR_L)$	C = 2	C = 3
0 < x < 1	86%	84%
1 < x < 2	9%	10%
2 < x < 3	3%	4%
3 < x < 4	0%	1%
4 < x	3%	1%
Max	4.75	4.18
Mean	0.41	0.56
Median	0.07	0.29

3.1.2. Varying the estimated number of contributors

3.1.2.1. Overestimation

The samples which were estimated to be two and three contributors' mixtures were also analyzed on both software assuming as having three and four contributors, respectively, so that the effect of overestimating the number of donors could be analyzed. On both of those variations, the verified trends were similar.

As can be seen on Figure 12, assuming a higher number of contributors than the one inferred by epg observation did not have much effect on the calculated LR. Although about 90% of the cases showed a decrease on the LRs computed in LRmix Studio (Table 10), these changes were slight: 61% were between one and two units on the log_{10} scale for mixtures of

two contributors analyzed as three; 86% within one unit for overestimate from three to four (Table 8). Introducing an additional donor to the analysis increases the possible genotype combinations; therefore, the genotype weights diffuse, consequently tending to lower the LR for the POI analyzed.

On quantitative model Euroformix, the impact of overestimation was even smaller. There was not observed an obvious trend in LRs variation - 58% of the obtained results with overestimation increased (Table 10) – and the great majority of the differences between the LRs calculated under the condition of overestimation and the LRs calculated with the first estimated number of donors were within one unit on the $\log_{10}$ scale (Table 8).

3.1.2.2. Underestimation

Samples which number of contributors was estimated as three were also analyzed assuming two donors. Oppositely to the latter experiment, in this one it was striking variations in a non-negligible proportion of cases. Figure 13 and Table 10 show how the underestimation led to a general decrease on the obtained LRs (mainly in quantitative model Euroformix). Although most of the differences in the calculated LRs were within two units on the $\log_{10}$ scale on both software - 68% for Euroformix and 76% for LRmix Studio – the former software registered some substantial declines on the LRs: 4% of the LRs computed by Euroformix were separated by more than 10 units on the $\log_{10}$ scale (Table 9). In fact, some of these cases were very distinct as they reduced their $\log_{10}$(LR) to negative values (LR dropped to values below one).

Accordingly, the effect of lowering the LR when reducing the estimated number of contributors was more emphasized in the quantitative model. This is likely to be explained by the alleles that become impossible to attribute to a contributor assuming an inferior number of donors. If there are peaks with small height (relatively to other alleles in the same marker) and in stutter position, the quantitative software considers it as unspecific (drop-in) or stutter; if those peaks correspond to alleles from the POI, the LR assigned by the software to that marker drops considerably.

To exemplify this, we present a particularly interesting case, exhibited in Figure 14. The quantitative model produced a nearly null LR (-73.61 in $\log_{10}$ scale) in a mixture with three estimated contributors, when two contributors were considered in the analysis. The obtained LR when analyzed with the estimated number of three contributors was 24.60, $\log_{10}$ scale. Specifically analyzing this case we noticed that the alleles of the reference sample corresponded to the alleles with smaller heights of the mixture. Withdrawing one contributor,

precisely the alleles with lower quantity of DNA are devaluated by the software. So, the software produces a result translating a residual probability of that reference profile being a contributor, given the observed mixture and under the assumption of two contributors.

As mentioned above, by lowering the estimate of number of contributors, some markers reveal a number of alleles that are not compatible with the new number of contributors defined, remaining alleles that cannot be explained as belonging to a donor. In the case where there are peaks that are not assigned but there are no peaks in stutter position nor very likely to be considered as drop-in (due to the drop-in probability or the peak height), it is assumed that the POI should not be a donor and a low LR is obtained as well. Figure 15 illustrates a marker representing one of these circumstances. It belongs to a mixture estimated to have three donors; analyzing it as a two contributors' mixture, the maximum allele number per marker should be four, representing a situation where all the contributors are heterozygotic. In this represented case, the POI is homozygotic; so, there is one allele remaining impossible to attribute to a contributor nor likely to be considered drop-in or stutter (due to the peak heights and positions). Hence, the software returns an extremely low LR value for such marker, which, due to the product rule, will condition the final numerical result.

The samples which dropped its log10(LR) to negative values are characterized by a relatively elevated number of alleles per mixture (4.24 to 3.95 alleles per locus, being the average in mixtures of three contributors of 3.71) and/or an elevated number of homozygotic loci in the POI profile (5 to 8 homozygous per profile, while the average of homozygous in all casework references is 4.13).

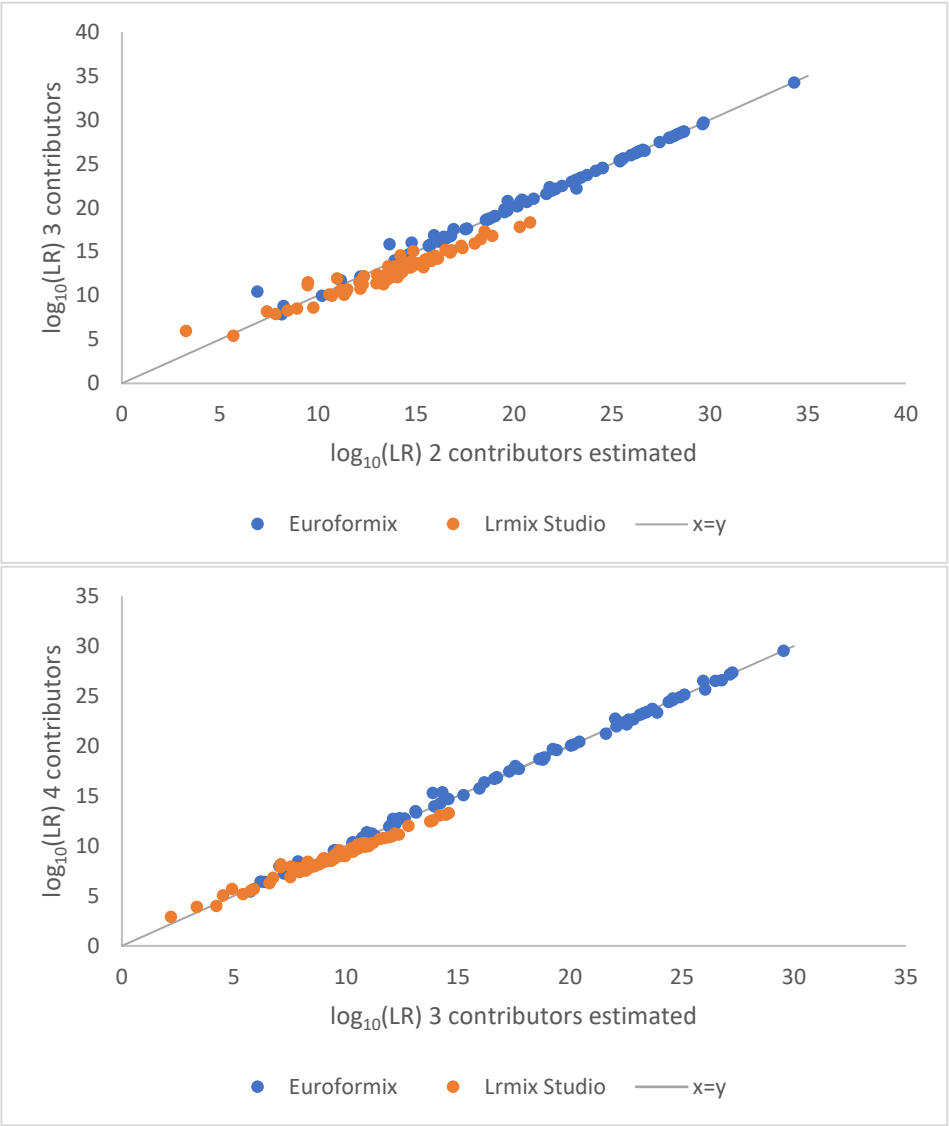


Figure 12. Plots showing the obtained $log_{10}(LR)$ through Euroformix (blue dots) and LRmix Studio (orange dots) when the number of contributors is overestimated. The upper plot represents the overestimation from two to three contributors; the lower plot represents the overestimation from three to four. The line represents $log_{10}(LR)$ estimated contributors = $log_{10}(LR)$ overestimation.

Table 8. Distribution of the differences between the LR (log_{10} scale) obtained with overestimating the number of contributors (from 2 to 3 and from 3 to 4) and with the initial estimate; and the maximum, mean, median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

$x=log_{10}(LR_H/LR_L)$	2 as 3		3 as 4	
	Euroformix	LRMix Studio	Euroformix	LRMix Studio
$0 < x < 1$	94%	29%	97%	86%
$1 < x < 2$	4%	61%	3%	14%
$2 < x < 3$	1%	10%	0%	0%
$3 < x$	1%	0%	0%	0%
Max	3.52	2.71	1.41	1.33
Mean	0.19	1.30	0.20	0.64
Median	0.01	1.39	0.11	0.64

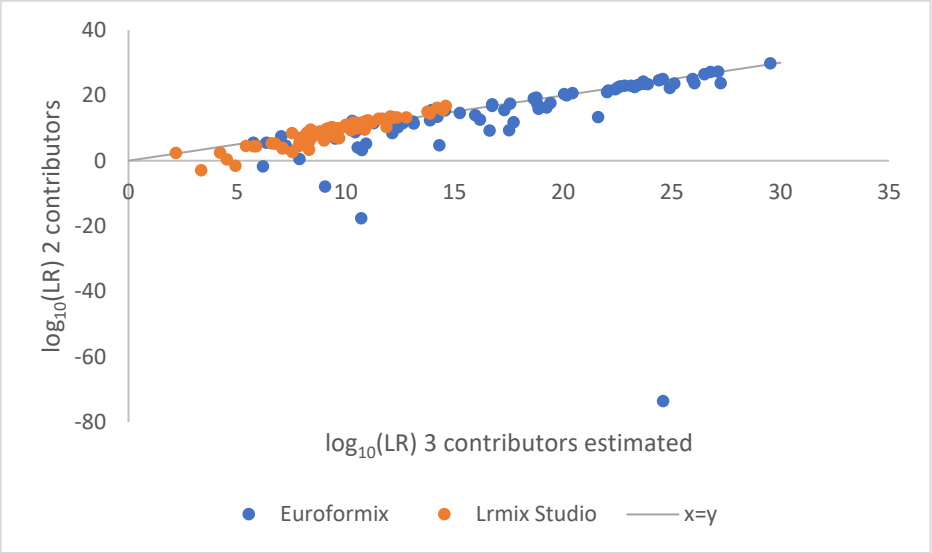


Figure 13. Plot showing the obtained log_{10}(LR) through Euroformix (blue dots) and LRmix Studio (orange dots) when the number of contributors is underestimated from three to two. The line represents log_{10}(LR) estimated contributors = log_{10}(LR) underestimation.

Table 9. Distribution of the differences between the LR (log_{10} scale) obtained with underestimating the number of contributors (from 3 to 2) and with the initial estimate; and the maximum, mean and median values of these differences. For simplicity purposes we considered the difference between the highest of the two LR (LR_H) and the lowest one (LR_L).

$x=log_{10}(LR_H/LR_L)$	3 as 2	
	Euroformix	LRMix Studio
$0 < x < 2$	68%	76%
$2 < x < 4$	15%	18%
$4 < x < 6$	1%	4%
$6 < x < 8$	6%	3%
$8 < x < 10$	5%	0%
$10 < x$	4%	0%
Max	98.22	6.49
Mean	3.66	1.44
Median	0.89	1.03

Table 10. Proportion of analyses that resulted in an increase or decrease of the LR when overestimating and underestimating the number of contributors, on both software.

		LR variation	
		Increase	Decrease
Overestimating	Euroformix	58%	42%
	LRmix Studio	11%	89%
Underestimating	Euroformix	32%	68%
	LRMix Studio	48%	52%

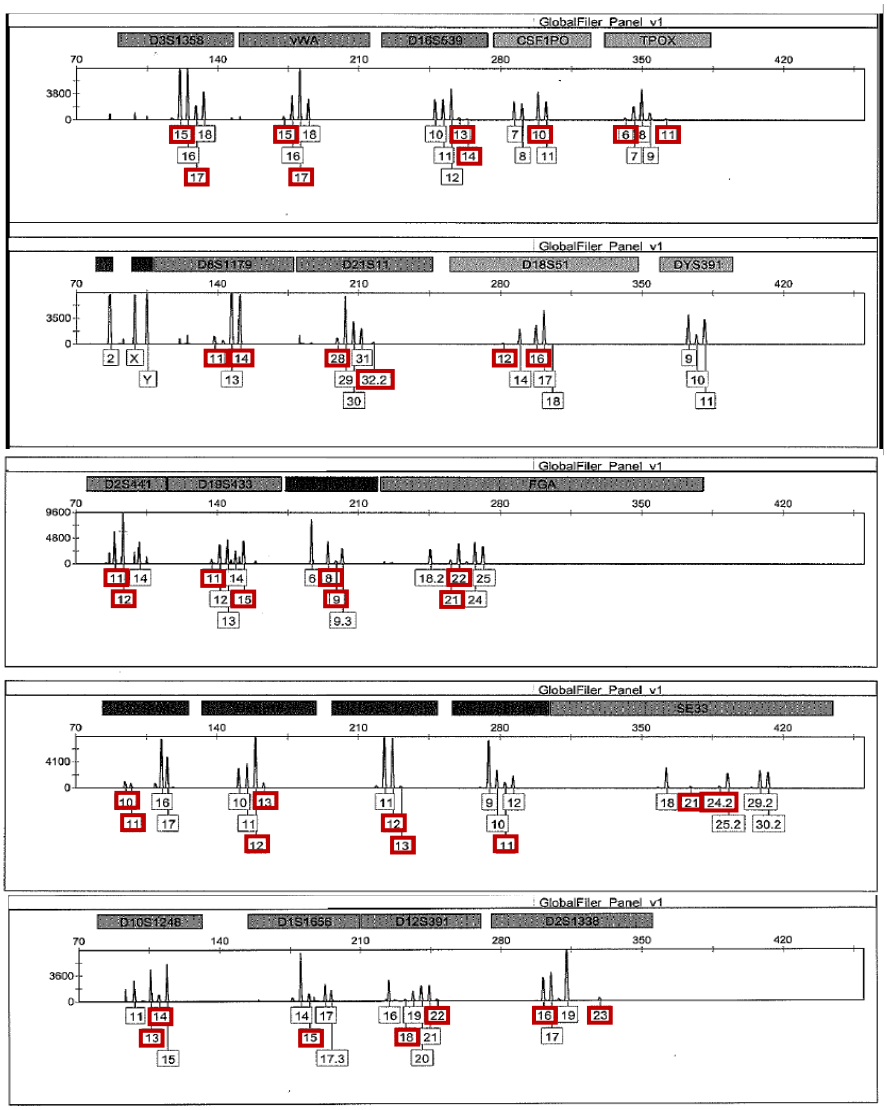


Figure 14. Case-example for a major alteration in the LR when the number of estimated contributors is lowered. The alleles highlighted in red correspond to matching alleles with the POI profile (all minor alleles).

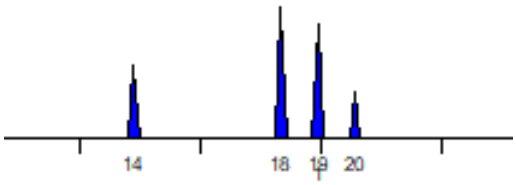


Figure 15. Representation of a marker with four alleles and where the POI is homozygotic (19). This exemplifies a situation where by decreasing the number of contributors (from three to two), alleles remain unexplained by the contributors and settings defined (hence lowering the LR).

3.2. Weighing the evidence with simulated references

3.2.1. With the estimated number of contributors

Barring mutation, a father-son duo shares at least one half of the total of their autosomal alleles. On the other hand, full-siblings share, with the same probability (0.25), none or two alleles originated in the same ancestral allele (IBD alleles). In this part of the work, for each pair mixture/reference, we simulated a full-sibling and a parent of the reference, based on his/her genotype and on the population allelic frequencies (Appendix I). Then, we computed the LR using these simulated profiles, on both software. That is, we calculated the likelihood of the observation of the mixture, assuming that the full-sibling or parent was a donor in the main hypothesis and unrelated with any contributor in the alternative one.

Although we are aware of the results on this experiment being biased, since the relatives' profiles were simulated from a profile that is not excluded to be a donor to the mixture, and the alternative hypothesis states that the reference is genetically unrelated of any donor of the mixture, we aimed to observe how the programs deal with cases where the reference is compatible only in some markers.

This experiment was carried out for each type of mixture (two and three contributors estimated) and for each software.

Figures 16 and 17 show the distribution of the computed LRs obtained for the simulated profiles of one full-sibling and one parent of the casework reference for each mixture (without changing the *a priori* hypotheses: the POI is a contributor of the mixture; and the POI is genetically unrelated to any donor of the mixture).

The obtained results were very similar varying the relative used as reference (Tables 11 and 12).

Comparing the results produced by the two software, some differences were found. The median of LRs produced by Euroformix was lower compared to the one obtained through LRmix Studio for mixtures with 2 contributors estimated, since the reference genotypes were simulated and often did not match likely genotypes assumed by the program based on peak heights. This trend was inversed for mixtures of 3 donors estimated, although with smaller discrepant results.

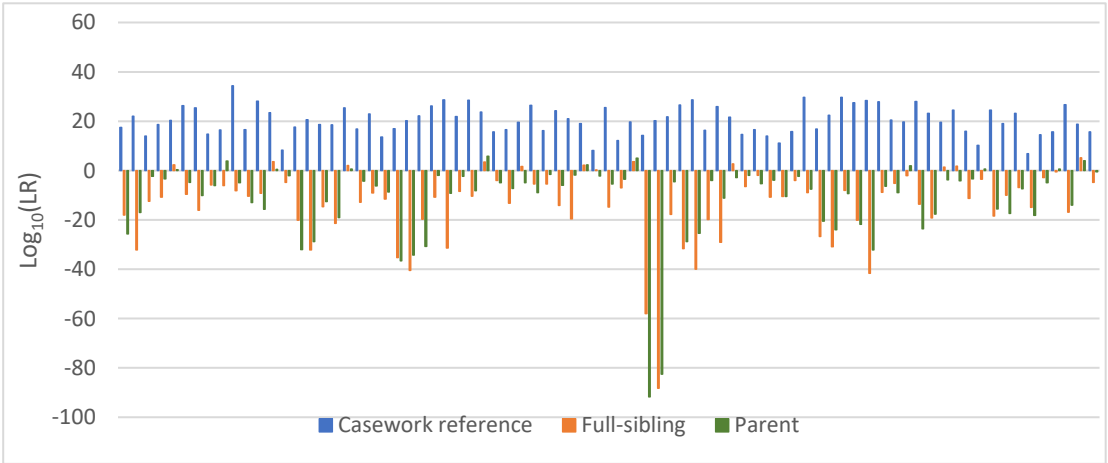
However, it was also observed that mixtures of three contributors estimated produced generally higher LRs comparing to those obtained for mixtures with two contributors estimated: not only produced more results with positive $\log_{10}$(LR), but also its negative

log_{10}(LR) are not as low as in assumed two person mixtures, as shown by Figures 16 and 17 and in Tables 11 and 12.

This indicates that more complex mixtures, here translated in number of estimated contributors, generally become less informative (and consequently, less discriminatory).

In the instances where the consideration of a full-sibling or a parent of the casework reference produced the higher LRs, the profiles generally had a great number of alleles in common with the casework reference (its relative); but not all the cases with higher amount of shared alleles produced higher LRs. Suppose that a shared allele between the full-sibling and the casework reference is present in the mixture. If this allele has a low frequency, this marker will be assigned with a high LR, influencing the total LR; although the full-sibling may have some mismatches with the mixture. Indeed, population allele frequencies have a great impact on the calculated LR. Take as example a specific case where considering a simulated full-sibling of the reference we obtained log_{10}(LR) = -35.36. It may be expected that that profile and the corresponding casework reference shared just a few alleles; however, the profiles share 31 out of 42 alleles; which is even more that the number of alleles shared between some full-sibling and casework references that produced the higher log_{10}(LR): for example, one of these samples (of a mixture of two estimated donors) produced a log_{10}(LR) = 3.63, sharing only 26 out of the 42 alleles with its corresponding real reference.

Notwithstanding, the positive log_{10}(LR) obtained in these analyses would not be a statistically strong result – all were situated below log_{10}(LR)=8.



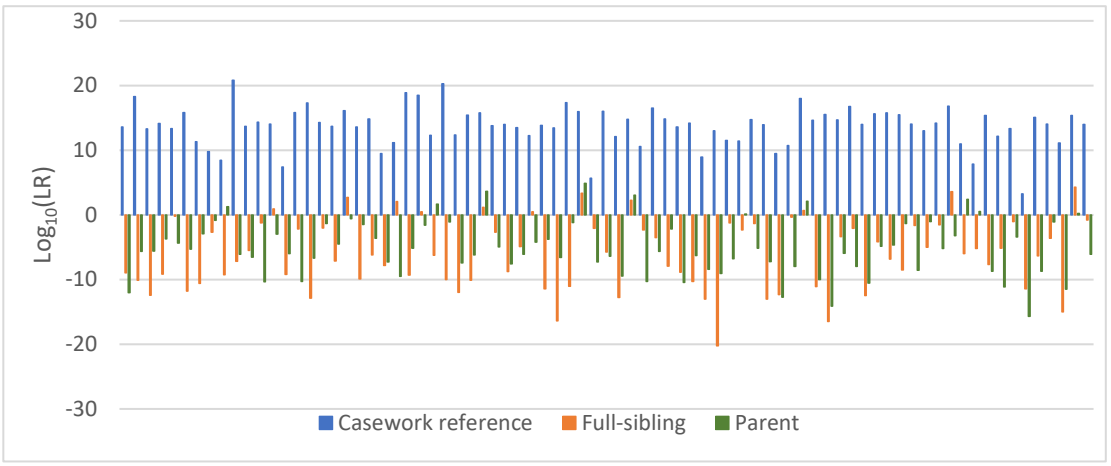


Figure 16. Computed $log_{10}(LR)$ for each of the mixtures with two estimated contributors for casework reference (blue bar), for a simulated full-sibling (orange bar) and for a simulated parent (green bar), in Euroformix (upper) and LRmix Studio (lower).

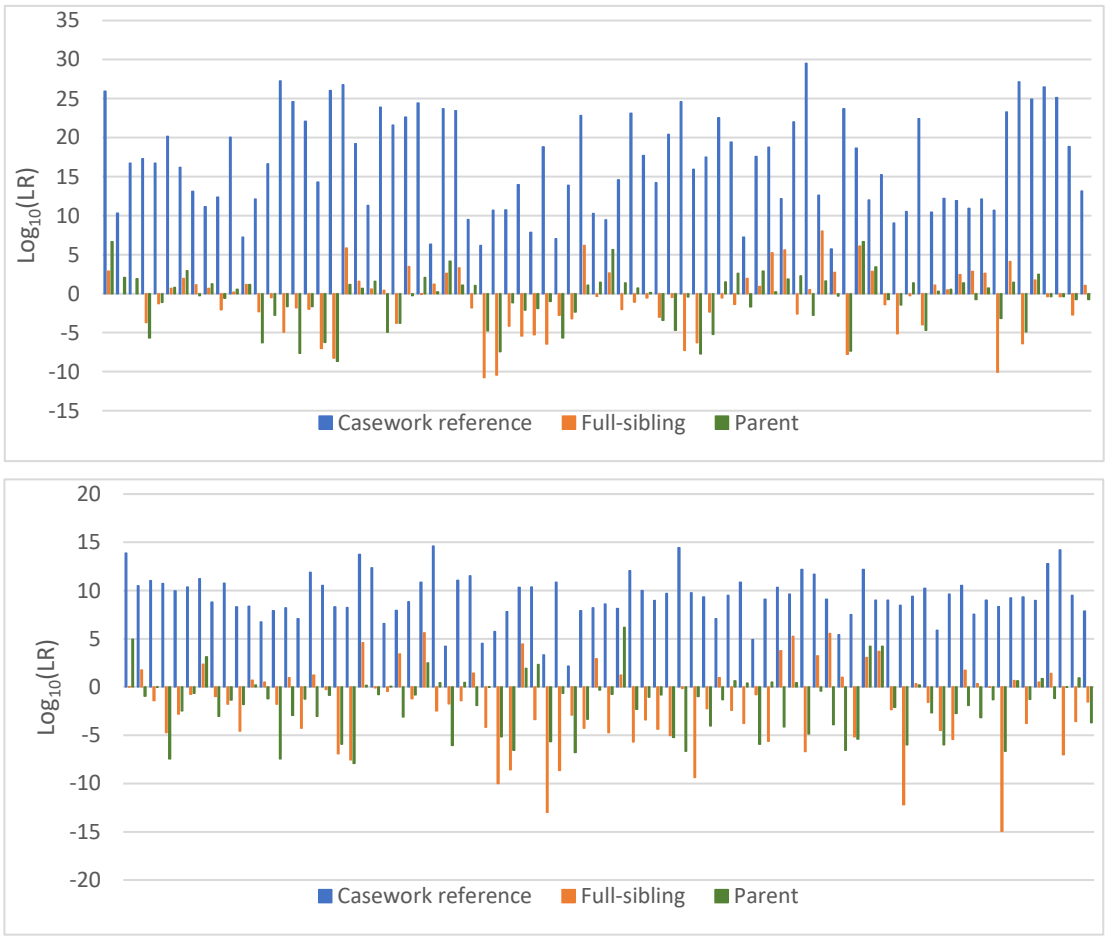


Figure 17. Computed $log_{10}(LR)$ of each of the mixtures with three estimated contributors for casework reference (blue bar), for a simulated full-sibling (orange bar) and for a simulated parent (green bar), in Euroformix (upper) and LRmix Studio (lower).

Table 11. Proportion of simulated profiles of full-siblings that produced a positive log_{10}(LR), the maximum log_{10}(LR) value, the total median and the median of positive log_{10}(LRs), on both software, for mixtures of C estimated contributors.

	C = 2		***C = 3***	
	Euroformix	***LRmix Studio***	***Euroformix***	***LRmix Studio***
Log_{10}(LR)>0	15%	14%	44%	35%
Max	5.14	4.27	8.03	5.62
Median	-10.47	-6.18	-0,39	-1.55
Median [if log_{10}(LR)>0]	2.24	2.08	1.95	1.59

Table 12. Proportion of simulated profiles of parents that produced a positive log_{10}(LR), the maximum log_{10}(LR) value, the total median and the median of positive log_{10}(LRs), on both software, for mixtures of C estimated contributors.

	C = 2		***C = 3***	
	Euroformix	***LRmix Studio***	***Euroformix***	***LRmix Studio***
Log_{10}(LR)>0	14%	13%	48%	27%
Max	5.75	4.88	6.68	6.20
Median	-6.10	-5.63	-0,28	-1.27
Median [if log_{10}(LR)>0]	1.94	1.90	1.42	0.66

3.2.2. Varying the estimated number of contributors

3.2.2.1. Overestimation

When considering an extra contributor beyond those estimated in the analysis (from three to four contributors), in both software and in both relative profiles tested, there was a general increase of the LRs (Table 15; Appendix IV) (oppositely to the trend verified with overestimation using real references, in LRmix Studio – see 3.1.2.1).

In Euroformix computer program, the greater part of the analyses produced positive log_{10}(LR) results, for both full-sibling and parent references (Tables 13 and 14).

Regarding the qualitative model LRmix Studio, it also revealed similar percentages for both types of relatives when overestimating the number of contributors: about 45% of the cases obtained a positive log_{10}(LR), for full-siblings and parents (Tables 13 and 14).

Although the majority of the positive log_{10}(LR) obtained may not be considered very informative - medians between 1.01 and 1.77 -, these results show that introducing a different number that the one inferred through the epg, in this case by increasing it, can potentially lead to a false inclusion, especially in circumstances like this, where a relative of a contributor is considered the POI.

3.2.2.2. Underestimation

Contrarily to the previously situation, reducing the number of contributors (from three to two) induced a general decline of the LRs (Appendix V). In fact, both quantitative and qualitative software responded equally, with all cases showing the decrease trend (Table 16). Also for both programs, the proportions of cases with positive log_{10}(LR) in this situation was very low (Tables 13 and 14).

Not only the amount of samples with a negative log_{10}(LR) increased (relatively to the ones obtained for full-siblings and parents assigning the estimated number of contributors by allele count), but also the result values dropped considerably.

By reducing the number of contributors, the possible genotype combinations drop as well, narrowing the possibility of the profile of the POI, that is a non contributor in this instance, to fit into the mixture.

Table 13. Proportion of simulated profiles of full-siblings that produced a positive log_{10}(LR) when the number of contributors was over- and underestimated, the maximum log_{10}(LR) value, the total median and the median of positive log_{10}(LRs), on both software.

	Overestimation (3 as 4)		***Underestimation (3 as 2)***	
	Euroformix	***LRmix Studio***	***Euroformix***	***LRmix Studio***
Log_{10}(LR)>0	81%	46%	9%	9%
Max	7.98	5.63	7.61	4.93
Median	1.06	-0.56	-13.75	-8.66
Median [if log_{10}(LR)>0]	1.59	1.77	3.02	2.35

Table 14. Proportion of simulated profiles of parents that produced a positive log_{10}(LR) when the number of contributors was over- and underestimated, the maximum log_{10}(LR) value, the total median and the median of positive log_{10}(LRs), on both software.

	Overestimation (3 as 4)		**Underestimation (3 as 2)**	
	Euroformix	**LRmix Studio**	**Euroformix**	**LRmix Studio**
Log_{10}(LR)>0	81%	44%	4%	5%
Max	7.03	5.90	5.82	4.69
Median	0.93	-0.46	-11.81	-8.11
Median [if log_{10}(LR)>0]	1.40	1.01	4.17	2.49

Table 15. Proportion of analyses that resulted in an increase or decrease of the LR when overestimating and underestimating the number of contributors using simulated full-sibling references, on both software.

		LR variation	
		Increase	***Decrease***
Overestimating	***Euroformix***	94%	6%
	LRmix Studio	91%	9%
Underestimating	***Euroformix***	0%	100%
	LRMix Studio	0%	100%

Table 16. Proportion of analyses that resulted in an increase or decrease of the LR when overestimating and underestimating the number of contributors using simulated parent references, on both software.

		LR variation	
		Increase	***Decrease***
Overestimating	***Euroformix***	92%	8%
	LRmix Studio	94%	6%
Underestimating	***Euroformix***	0%	100%
	LRMix Studio	0%	100%

4. Conclusions

Existing software programs of interpretation of forensic samples are invaluable tools for applying the mathematical models implied to the computation of a probative value. This calculation is able to account for several parameters, regarding population and analytical factors, on which the LR depends on and which are introduced by the user. Naturally, many forensic laboratories adopt default values and the entered values are subjected to errors.

Complexity increases in the cases where the genetic evidence contains a mixture of profiles, where the number of sources who contributed to the sample must be estimated and introduced in the software by the user. Since this is a parameter generally empirically estimated by an expert, it is also comprehensible that different persons can have a different interpretation on this regard, in more challenging samples.

Accordingly, it is important to know in which extent variations in those parameters can affect the statistical evaluation of the genetic evidence (measured via LRs). It is also essential to be aware of the differences that different types of software can yield on the computed results.

Mixtures with different number of contributors estimated were included in this work, showing differences in the general computed LRs, being that higher order mixtures (three estimated contributors) generated lower LRs than estimated two contributors' mixtures. The more estimated contributors for a mixture, the less powerful probative value achieved.

Overall, the variation of the parameters: co ancestry coefficient of the population, allele drop-in and allele detection threshold, did not impact LR in a substantial form. The exceptional cases in which LR was more affected were: when altering the drop-in probability to zero in the quantitative program; when varying the F_{ST} value in evidences containing rare alleles matching with the reference; when increasing the threshold limit discarded several alleles that would match between the evidence and the reference.

The variation of the referred parameters influenced in similarly mixtures with two and three estimated donors.

Varying the number of contributors of a mixture had little to moderate effect, in general. However, overestimation led to a slight decrease trend in qualitative software LRmix Studio; more significantly, the LR results of a few samples suffered a great impact when underestimating this parameter in quantitative model Euroformix, potentially leading to a different interpretation of the final result. This reinforces that the previous interpretation of the expert, where the number of contributors is estimated is, indeed, a crucial step in forensic mixtures analyses.

Drop-in and F_{ST} were the other analyzed parameters in both programs. In these variations, the LRs were similarly influenced in two programs, except when drop-in was considered as null, in which case programs produced more differentiating results, since Euroformix computed much lower LRs.

As expected, the quantitative model generally produces stronger results (higher LRs), since it integrates more information of the electropherogram on its calculations.

A situation that may occur in casework forensics is the sampling of a reference that is, in fact, relative to a contributor to the evidence, being the expert unaware of this fact. Unsurprisingly, the LRs decreased when considering a simulated profile of 1st degree relatives of the casework reference. However, some of the results were fairly elevated for a non-contributor, which we know is due to the familiar link to a person that cannot be excluded from have contributed to the mixture; but which in casework context could be an inconclusive result.

For the case of simulated profiles of relatives of the real reference, much more samples of mixtures with three donors estimated produced a positive $\log_{10}$(LR) (and higher LRs as well), comparing to those obtained with mixtures with two donors estimated. For estimated three donors' mixtures, some computed LRs for simulated references were higher than LRs obtained with casework references. Precisely, the maximum LR obtained for a simulated parent in Euroformix was higher than 9% of the LRs obtained for casework references (regarding three donors' mixtures). The same situation was verified for 10% of the LRs calculated on the qualitative model.

On the other hand, the overestimation of the number of donors of the mixture and the consideration of simulated references, led Euroformix to produce positive log10(LRs) in about the double of the cases obtained through LRmix Studio.

Globally, the results obtained in this work show that a software based in the quantitative model can be more effective in assist in more complex interpretations, comparing to a qualitative model. Nevertheless, it presupposes a correct and meticulous analysis from the very beginning of the process, i.e., from the evidence collection, to the epg analysis, so that possible errors are minimized. A well collected sample (maximizing the genetic material subjected to analysis and minimizing the possible sources of contamination) provides a profile easier to interpret. Consequently, the decisions that the expert must ensure about some presented peaks (belonging to a donor, artefacts or stochastic effects) and the estimation of the number of contributors will be optimized and, finally, the software interpretation will be more reliable. It is worth mention that the experts experience and knowledge have a central importance, since his interpretation will affect the software computation interpretation.

5. References

1. Watson, J.D. & Crick, F.H.C. (1953). A Structure for Deoxyribose Nucleic Acid. Nature, 171, 737-738.
2. Goodwin, W., Linacre, A. & Hadi, S. (2011). An introduction to forensic genetics. John Wiley & Sons.
3. Butler, J.M. (2010). Fundamentals of Forensic DNA Typing. Elsevier.
4. International Human Genome Sequencing Consortium (2004). Finishing the euchromatic sequence of the human genome. Nature, 431(7011), 931-945.
5. Schneider, P.M. (1997) Basic issues in forensic DNA typing. Forensic Science International, 88(1), 17-22.
6. Amorim, A. & Budowle, B. (2016). Handbook of forensic genetics: Biodiversity and Heredity in Civil and Criminal Investigation. World Scientific.
7. Jeffreys, A.J., Allen, M.J., Armour, J.A.L., Collick, A., Dubrova, Y., Fretwell, N., Guram, T., Jobling, M., May, C.A., Nell, D.L., Neumann, R. (1995). Mutation processes at human minisatellites. Electrophoresis, 16(1), 1577-1585.
8. Jeffreys, A.J., Wilson, V., Neumann, R. & Keyle, J. (1988). Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells. Nucleic Acids Research, 16(23), 10953-10971.
9. Schlötterer, C. (2000). Evolutionary dynamics of microsatellite DNA. Chromosoma, 109(6), 365-371.
10. Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics, 5(6), 435-445.
11. Weber, J.L. & Wong, C. (1993). Mutation of human short tandem repeats. Human Molecular Genetics, 2(8), 1123-1128.
12. Schneider, P.M., Blender, K., Mayr W.R., Parson W., Hoste, B., Decorte, R., Cordonnier, J., Vanek, D., Morling, N., Schmitter H., Pflung, W., Wenzel, R., Patzelt, D., Lessig, R., Dobrowolski, P., O'Donnell G., Garafano, L., Dobosz, M., Knijff, P., Mevag, B., Pawlowski, R., Gusmão, L., Vide, M.C., Alonso, A.A., Fernández, O.G., Teyssier, A., Coquoz, R., Brandt, C., Germann, U., Gill, P., Hallett, J. & Greenhalgh M. (2004) STR analysis of artificially degraded DNA—results of a collaborative European exercise. Forensic Science International, 139(2-3), 123-134.
13. The 1000 Genomes Project Consortium (2015). A global reference for human genetic variation. Nature, 526(7571), 68-74.

14. Gill, P. (2001). An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. International Journal of Legal Medicine, 114(4-5), 204-210.
15. Børsting, C., Mogensen, H.S. & Morling, N. (2013). Forensic genetic SNP typing of low-template DNA and highly degraded DNA from crime case samples. Forensic Science International: Genetics, 7(3), 345-352.
16. Phillips, C., Fondevila, M., García-Magariños, M., Rodriguez, A., Salas, A., Carracedo, Á. & Lareu M.V. (2008). Resolving relationship tests that show ambiguous STR results using autosomal SNPs as supplementary markers. Forensic Science International: Genetics, 2(3), 198-204.
17. Pinto, N., Magalhães. M., Conde-Sousa, E., Gomes, C., Pereira, R., Alves, C., Gusmão, L. & Amorim, A., Assessing paternities with inconclusive STR results: the suitability of bi-allelic markers, Forensic Science International: Genetics (2013) 7: 16-21.
18. Phillips, C., Salas, A., Sánchez, J.J., Fondevila, M., Gómez-Tato, A., Álvarez-Dios, J., Calaza, M., Casares de Cal, M., Ballard, D., Lareu M.V. & Carracedo, Á. (2007). Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. Forensic Science International: Genetics, 1(3-4), 273-280.
19. Nachman, M.W. & Crowell, S.L. (2000). Estimate of the Mutation Rate per Nucleotide in Humans. Genetics, 156(1), 297-304.
20. Bastos-Rodrigues, L., Pimenta, J.R. & Pena S.D.J. (2006). The Genetic Structure of Human Populations Studied Through Short Insertion-Deletion Polymorphisms. Annals of Human Genetics, 70(5), 658-665.
21. Pereira, R., Phillips, C., Pinto, N., Santos, C., Santos, S.E.B., Amorim, A., Carracedo, Á. & Gusmão, L. (2012). Straightforward Inference of Ancestry and Admixture Proportions through Ancestry-Informative Insertion Deletion Multiplexing. PLoS ONE, 7(1), e29684.
22. Pereira, R., Phillips, C., Alves, C., Amorim, A., Carracedo, Á. & Gusmão, L. (2009). A new multiplex for human identification using insertion/deletion polymorphisms. Electrophoresis, 30(21), 3682-3690.
23. Jeffreys, A.J., Wilson, V. & Thein. S.L. (1985). Hypervariable "minisatellite" regions in human DNA. Nature, 314(6006), 67-73.
24. Jeffreys, A.J., Wilson, V. & Thein. S.L. (1985). Individual-specific "fingerprints" of human DNA. Nature, 316(6023), 76-79.
25. Jeffreys, A.J., Brookfield, J.F.Y. & Semeonoff, R. (1985). Positive identification of an immigration test-case using human DNA fingerprints. Nature, 317(6040), 818-819.
26. Roewer, L. (2013). DNA fingerprinting in forensics: past, present, future. Investigative genetics, 4(1), 22.

27. Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro The polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology, 51, 263-273.
28. Edwards, A., Civitello, A., Hammond, H.A. & Caskey, T. (1991). DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. American journal of human genetics, 49(4), 746-756.
29. Wallin, J.M., Buoncristiani, M.R., Lazaruk, K.D., Fildes, N., Holt, C.L. & Walsh, P.S. (1998). TWGDAM Validation of the AmpFISTR Blue PCR Amplification Kit for Forensic Casework Analysis. Journal of Forensic Sciences, 43(4), 854-870.
30. Wang, D.Y., Gopinath, S., Lagacé, R.E., Norona, W., Henessy, L.K., Short, M.L. & Mulero, J.J. (2015). Developmental validation of the GlobalFiler® Express PCR Amplification Kit: A 6-dye multiplex assay for the direct amplification of reference samples. Forensic Science International: Genetics, 19, 148-155.
31. Jobling, M.A & Gill, P. (2004). Encoded evidence: DNA in forensic analysis. Nature Reviews Genetics, 5(10), 739-751.
32. Vandenberg, N. & van Oorschot, R.A.H. (2006). The Use of PolilightR in the Detection of Seminal Fluid, Saliva, and Bloodstains and Comparison with Conventional Chemical-Based Screening Tests. Journal of Forensic Sciences, 51(2), 361-370.
33. Webb, J.L., Creamer, J.I. & Quickenden, I. (2006). A comparison of the presumptive luminol test for blood with four non-chemiluminescent forensic techniques. Luminescence, 21(4), 214-220.
34. Tobe, S.S., Watson, N. & Daéid N.N. (2007). Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecular-Weight DNA. Journal of Forensic Sciences, 52(1), 102-109.
35. Park, S.-M., Park, S.-Y., Kim, J.-H., Kang, T.-W., Park, J.-L., Woo, K.-M., Kim, J.-S., Lee, H.-C., Kim, S.-Y. & Lee, S.-H. (2013). Genome-wide mRNA profiling and multiplex quantitative RT-PCR for forensic body fluid identification. Forensic Science International: Genetics, 7(1), 143-150.
36. Courts, C. & Madea, B. (2011). Specific Micro-RNA Signatures for the Detection of Saliva and Blood in Forensic Body-fluid Identification. Journal of Forensic Sciences, 56(6), 1464-1470.
37. Lee, H.Y., Park, M.J., Choi, A., An, J.H., Yang, W.I. & Shin, K.-J. (2012). Potential forensic application of DNA methylation profiling to body fluid identification. International Journal of Legal Medicine, 126(1), 55-62.

38. Hochmeister, M.N., Budowle, B., Borer, U.V., Eggmann, U., Comey, C.T. & Dirnhofer, R. (1991). Typing of deoxyribonucleic acid (DNA) extracted from compact bone from human remains. Journal of Forensic Sciences, 36(6), 1649-61.
39. Walsh, P.S., Metzger, D.A. & Higuchi, R. (1991). Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material. BioTechniques, 10(4), 506-513.
40. Wang, D.Y., Chang, C.-W., Oldroyd, N.J. & Henessy, L.K. (2009). Direct amplification of STRs from blood or buccal cell samples. orensic Science International: Genetics Supplement Series, 2(1), 113-114.
41. Montpetit, S.A., Fitch, I.T. & O'Donnell, P.T. (2005). A Simple Automated Instrument for DNA Extraction in Forensic Casework. Journal of Forensic Sciences, 50(3), 1-9.
42. Liu, J.Y., Zhong, C., Holt, A., Lagace, R, Harrold, M., Dixon, A.B., Brevnov, M.G., Shewale, J.G. & Hennessy, L.K. (2012). AutoMate Express™ Forensic DNA Extraction System for the Extraction of Genomic DNA from Biological Samples. Journal of Forensic Sciences, 57(4), 1022-1030.
43. Gill. P., Jeffreys, A.J. & Werrett, D.J. (1985). Forensic application of DNA 'fingerprints'. Nature, 318(6046), 577-579.
44. Nicklas, J.A. & Buel, E. (2003). Quantification of DNA in forensic samples. Analytical and Bioanalytical Chemistry, 376(8), 1160-1167.
45. Walsh, P.S, Varlaro, J. & Reynolds, R. (1992). A rapid chemiluminescent method for quantitation of human DNA. Nucleic Acids Research, 20(19), 5061-5065.
46. Mandrekar, M.N., Erickson, A.M., Kopp, K., Krenke, B.E., Mandrekar, P.V., Nelson, R., Peterson, K., Shultz, J., Tereba, A. & Westphal, N. (2001). Development of a Human DNA Quantitation System. Forensic Sciences, 42(3), 336-339.
47. Higuchi, R., Dollinger, G., Walsh, P.S. & Griffith, R. (1992). Simultaneous Amplification and Detection of Specific DNA Sequences. Bio/Technology, 10(4), 413-417.
48. Higuchi, R., Fockler, C., Dollinger, G. & Watson, R. (1993). Kinetic PCR analysis: Real-time monitoring of DNA amplification reactions. Nature Biotechnology, 11(9), 1026-1030.
49. Hudlow, W.R., Chong, M.D., Swango, K.L., Timken, M.D. & Buoncristiani, M.R. (2008). A quadruplex real-time qPCR assay for the simultaneous assessment of total human DNA, human male DNA, DNA degradation and the presence of PCR inhibitors in forensic samples: A diagnostic tool for STR typing. Forensic Science International: Genetics, 2(2), 108-125.
50. Wilhelm, J. & Pingoud, A. (2003). Real-Time Polymerase Chain Reaction. ChemBioChem, 4(11), 1120-1128.

51. Chamberlain, J.S., Gibbs, R.A., Ranier, J.E., Nguyen, P.N & Thomas, C. (1988). Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Research, 16(23), 11141-11156.
52. Krenke, B.E., Tereba, A., Anderson, S.J., Buel, E., Culhane, S., Finis, C.J., Tomsey, C.S., Zachetti, J.M., Masibay, A., Rabbach, D.R., Amiott, E.A. & Sprecher, C.J. (2002). Validation of a 16-Locus Fluorescent Multiplex System. Journal of Forensic Sciences, 47(4), 773-785.
53. Sprecher, C.J., Puers, C., Lins, A.M. & Schumm, J.W. (1996). General Approach to Analysis of Polymorphic Short Tandem Repeat Loci. BioTechniques, 20, 266-276.
54. Lazaruk, K., Walsh, P.S., Gibert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C. & Wallin, J. (1998). Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument, Electrophoresis, 19, 86-93.
55. Butler, J.M., Buel, E., Crivellente, F. & McCord, B.R. (2004). Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. Electrophoresis, 25(1011), 1397-1412.
56. Buckleton, J.S., Bright, J.-A. & Taylor, D. (2016). Forensic DNA Evidence Interpretation. 2nd Edition, CRC Press.
57. Rakay, C.A., Bregu, J. & Grgicak, C.M. (2012). Maximizing allele detection: Effects of analytical threshold and DNA levels on rates of allele and locus drop-out. Forensic Science International: Genetics, 6(6), 723-728.
58. Gill, P., Puch-Solis, R. & Curran, J. (2009). The low-template-DNA (stochastic) threshold—Its determination relative to risk analysis for national DNA databases. Forensic Science International: Genetics, 3(2), 104-111.
59. Walsh, P.S., Fildes, N.J. & Reynolds, R. (1996). Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. Nucleic Acids Research, 24(14), 2807-2812.
60. Gibb, A.J., Huell, A.L., Simmons, M.C. & Brown, R.M. (2009). Characterisation of forward stutter in the AmpFlSTR® SGM Plus® PCR. Science & Justice, 49(1), 24-31.
61. Güixens-Gallardo, P., Hocek, M. & Perlíková, P. (2016). Inhibition of non-templated nucleotide addition by DNA polymerases in primer extension using twisted intercalating nucleic acid modified templates. Bioorganic & Medicinal Chemistry Letters, 26(2), 288-291.
62. Butler, J.M., Shen, Y. & McCord, B.R. (2003). The development of reduced size STR amplicons as tools for analysis of degraded DNA. Journal of Forensic Sciences, 48(5), 1054-1064.

63. Budowle, B, Eisenberg, A.J. & van Daal, A. (2009). Validity of Low Copy Number Typing and Applications to Forensic Science. Croatian Medical Journal, 50(3), 207-217.
64. Gill, P. Whitaker, J., Flaxman, C, Brown, N. & Buckleton, J. (2000). An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. Forensic Science International, 112(1), 17-40.
65. Whitaker, J.P., Cotton, E.A. & Gill, P. (2001). A comparison of the characteristics of profiles produced with the AMPFlSTR SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis. Forensic Science International, 123(2-3), 215-223.
66. Pfeifer, C.M., Klein-Unseld, R., Klintschar, M & Wiegand, P. (2012). Comparison of different interpretation strategies for low template DNA mixtures. Forensic Science International: Genetics, 6(6), 716-722.
67. Buckleton, J. (2009). Validation issues around DNA typing of low level DNA. Forensic Science International: Genetics, 3(4), 255-260.
68. Børsting, C., Mogensen, H.S. & Morling, N. (2013). Forensic genetic SNP typing of low-template DNA and highly degraded DNA from crime case samples. Forensic Science International: Genetics, 7(3), 345-352.
69. Hill, C.R., Kline, M.C, Coble, M.D. & Butler, J.M. (2007). Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples. Journal of Forensic Sciences, 53(1), 73-80.
70. Senge, T., Madea, B., Junge, A., Rothschild, M.A. & Schneider, P.M. (2011). STRs, mini STRs and SNPs – A comparative study for typing degraded DNA. Legal Medicine, 13(2), 68-74.
71. Gill, P., Brenner, C.H., Buckleton, J.S., Carracedo, A., Krawczak, M., Mayr, W.R., Morling, N., Prinz, M., Schneider, P.M. & Weir, B.S. (2006). Recommendations on the interpretation of mixtures. Forensic Science International, 160, 90-101.
72. Clayton, T.M., Whitaker, J.P., Sparkes, R. & Gill, P. (1998). Analysis and interpretation of mixed forensic stains using DNA STR profiling. Forensic Science International, 91(1), 55-70.
73. Benschop, C.C.G., Haned, H., Jeurissen, L., Gill, P.D. & Sijen, T. (2015). The effect of varying the number of contributors on likelihood ratios for complex DNA mixtures. Forensic Science International: Genetics, 19, 92-99.
74. Gill, P. Haned, H. (2013). A new methodological framework to interpret complex DNA profiles using likelihood ratios. Forensic Science International: Genetics, 7(2), 251-263.
75. Haned, H., Pène, L., Lobry, J.R., Dufour, A.B. & Pontier, D. (2011). Estimating the Number of Contributors to Forensic DNA Mixtures: Does Maximum Likelihood Perform Better Than Maximum Allele Count?. Journal of Forensic Sciences, 56(1), 23-28.

76. Biedermann, A., Bozza, S., Konis, K. & Taroni, F. (2012). Inference about the number of contributors to a DNA mixture: Comparative analyses of a Bayesian network approach and the maximum allele count method. Forensic Science International: Genetics, 6(6), 689-696.
77. Tvedebrink, T. (2014). On the exact distribution of the numbers of alleles in DNA mixtures, International Journal of Legal Medicine. 128(3), 427-437.
78. Swaminathan, H., Grgicak, C.M., Medard, M. & Lun, D.S. (2015). NOCIt: A computational method to infer the number of contributors to DNA samples analyzed by STR genotyping. Forensic Science International: Genetics, 16, 172-180.
79. Bright., J.-A., Richards, R., Kruijver, M., Kelly, H., McGovern, C., Magee, A., McWhorter, A., Ciecko, A., Peck, B., Baumgartner, C., Buettner, C., McWilliams, S., McKenna, C., Gallacher, C., Mallinder, B., Wright, D., Johnson, D., Catella, D., Lien, E., O'Connor, C., Duncan, G., Bundy, J., Echard, J., Lowe, J., Stewart, J., Corrado, K., Gentile, S., Kaplan, M., Hassler, M., McDonald, N., Hulme, P., Oefelein, R.H., Montpetit, S., Strong, M., Noël, S., Malsom, S., Myers, S., Welti, S., Moretti, T., McMahon, T., Grill, T., Kalafut, T., Greer-Ritzheimer, M., Beamer, V., Taylor, D.A. & Buckleton, J.S. (2018). Internal validation of STRmix™ – A multi laboratory response to PCAST. Forensic Science International: Genetis, 34, 11-24.
80. Amorim, A. (2012). Opening the DNA black box: demythologizing forensic genetics. New Genetics and Society, 31(3), 259-270.
81. Curran, J.M., Triggs, C.M. & Buckleton, J. (1999). Interpreting DNA Mixtures in Structured Populations. Journal of Forensic Sciences, 44(5), 987-995.
82. Meester, R. & Sjerps M. (2004). Why the effect of prior odds should accompany the likelihood ratio when reporting DNA evidence. Law, Probability and Risk, 3(1), 51-62.
83. Balding, D.J. & Donnelly, P. (1994). The Prosecutor's Fallacy and DNA Evidence. Criminal Law Review, 1, 711-721.
84. Gill, P., Gusmão, L., Haned, H., Mayr, W.R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P.M. & Weir, B.S. (2012). Recommendations on the evaluation of STR typing results that may include drop-out and / or drop-in using probabilistic methods. Forensic Science International: Genetics, 6, 679-688.
85. Buckleton, J. & Curran, J. (2008). A discussion of the merits of random man not excluded and likelihood ratios. Forensic Science International: Genetics, 2(4), 343-348.
86. Kelly, H., Bright, J.-A., Buckleton, J.S. & Curran, J.M. (2014). A comparison of statistical models for the analysis of complex forensic DNA profiles. Science & Justice, 54(1), 66-70.
87. Haned, H., Gill, P, Lohmueller, K., Inman, K. & Rudin, N. (2016). Validation of probabilistic genotyping software for use in forensic DNA casework: Definitions and illustrations. Science & Justice, 56(2), 104-108.

88. Gill, P. Kirkham, A. & Curran, J. (2007). LoComatioN: A software tool for the analysis of low copy number DNA profiles. Forensic Science International, 166(2-3), 128-138.
89. Haned, H., Slooten, K. & Gill, P. (2012). Exploratory data analysis for the interpretation of low template DNA mixtures. Forensic Science International: Genetics, 6(6), 762-774.
90. Cowell, R.G., Lauritzen, S.L. & Mortera, J. (2011). Probabilistic expert systems for handling artifacts in complex DNA mixtures. Forensic Science International: Genetics, 5(3), 202-209.
91. Bleka, Ø., Storvik, G. & Gill, P. (2016). EuroForMix : An open source software based on a continuous model to evaluate STR DNA profiles from a mixture of contributors with artefacts. Forensic Science International: Genetics, 21, 35-44.
92. Norsworthy, S., Lun, D.S. & Grgicak, C.M. (2018). Determining the number of contributors to DNA mixtures in the low-template regime: Exploring the impacts of sampling and detection effects. Legal Medicine, 32, 1-8.
93. Bright, J.-A., Curran, J.M. & Buckleton, J.S. (2014). The effect of the uncertainty in the number of contributors to mixed DNA profiles on profile interpretation. Forensic Science International: Genetics, 12, 208-214.
94. Curran, J.M., Buckleton, J.S. & Triggs, C.M. (2003). What is the magnitude of the subpopulation effect?. Forensic Science International, 135(1), 1-8.
95. Balding, D.J. & Nichols, R.A. (1994). DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands. Forensic Science International, 64(2-3), 125-140.
96. Puch-Solis, R. (2014). A dropin peak height model. Forensic Science International: Genetics, 11(1), 80-84.
97. Haned, H., Benschop, C.C.G., Gill, P.D. & Sijen, T. (2015). Complex DNA mixture analysis in a forensic context: Evaluating the probative value using a likelihood ratio model. Forensic Science International: Genetics, 16, 17-25.
98. Taylor, D., Bright, J.-A., Buckleton, J. & Curran, J. (2014). An illustration of the effect of various sources of uncertainty on DNA likelihood ratio calculations. Forensic science international. Genetics, 11, 56-63. +
99. Buckleton, J., Curran, J., Goudet, J., Taylor, D., Thiery, A. & Weir, B.S. (2016). Population-specific FST values for forensic STR markers: A worldwide survey. Forensic Science International: Genetics, 23, 91-100. +
100. Budowle, B. & Chakraborty, R. (2001). Population variation at the CODIS core short tandem repeat loci in Europeans. Legal Medicine, 3(1), 29-33.
101. Wolanska-Nowak, P. (2000). Application of subpopulation theory to evaluation of DNA evidence. Forensic Science International, 113(1-3), 63-69.

102. Gill, P., Foreman, L., Buckleton, J.S., Triggs, C.M. & Allen, H. (2003). A comparison of adjustment methods to test the robustness of an STR DNA database comprised of 24 European populations. Forensic Science International, 131(2-3), 184-196.

Appendices

Appendix I

Table 1. Allele frequencies of 23 autosomal markers for Caucasian population.

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D2S1045	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	SE33	TH01	TPOX	vWA
5																		0.0013850	0.0013850	0.0013850	0.0013850
6																		0.2354570	0.0013850	0.4155124	0.6502770
6.3																					
7														0.0027700	0.0027700	0.0831024	0.9307		0.1939058	0.1741745	0.1500000
8	0.0055401	0.6620498	0.1204986	0.1800554	0.0166205									0.0055401	0.1440443	0.0138504	0.1551246	0.0955678	0.5249307	0.4792243	0.8310249
8.1																0.0013850	0.4155124				
9	0.0138504	0.1551246		0.0756230	0.1066481	0.9944598	3							0.0013850	0.4155124	0.0415512	0.1675900	0.1191135	0.1274238	0.2271468	1
9.1														0.0013850	0.4155124	0.0554016	0.6620498				
9.3																		0.3448753	0.4626038	8	
10	0.2202216	0.0664819	0.0470914	0.0567867	0.0083102	0.0013850	0.0027700	0.0831024	9907				0.2105263	1.5789474	0.0554016	0.2562326	0.1024930	0.0083102	0.0498614	9584487	53
10.1																					
10.2																					
10.3																					
11	0.3088642	0.0013850	0.4155124	65	0.3254847	0.3144044	0.0096952	0.0055401	0.0775623	26869806	1	0.1398891	966759	0.3434903	0.0013850	0.3559556	0.2049861	0.0761772	8531855	96	0.0013850	0.0013850	0.2520775	6232687
11.2																					
11.3														0.0609418	2825484	76					
12	0.3601108	0.0318559	5567867	04	0.2686980	0.3144044	0.1135734	0.0706371	0.1163434	9030470	9	0.0124653	7396121	88	0.0470914	1.2742382	3.8781160	1.5927970	1.6759002	7700831	0.0069252	0.0775623	26	0.0415512	0.0013850
12.2									0.0013850	4155124	654										
12.3														0.0041551	2465373	961					
13	0.0817174	0.3074792	2437673	1	0.1163434	0.1634349	0.1232686	0.2548476	0.0664819	9445983	38	0.0069252	0775623	26	0.0290858	0.0013850	0.1426592	0.0346260	0.3296398	9196676	0.0166204	9861495	84	0.0013850	0.0013850
13.2									0.0069252	0775623	269										
13.3																					
13.4																					
14	0.0096952	0.2977839	3351800	6	0.0429362	0.0263157	0.1343490	0.3614958	0.0789473	6842105	26	0.0567867	0360110	8	0.2409972	0.1066481	0.0069252	0.0041551	0.1662049	8614958	4	0.0249307	4792243	77	0.0927977
14.2									0.0013850	0.0235457	0637119	11												0.0027700	8310249	307	
14.3									0.0027700	8310249	307										
15	0.1966759	0.0318559	0.0013850	4155124	65	0.1703601	0.1565096	0.1495844	8753460	6	0.3213296	0.0013850	0.0595567	0.2728531	0.0013850	4155124	65	0.1038781	1634349	0.0401662	0498614	96	0.1052631
15.2						0.0360110	8033241														
15.3							0.0581717	4515235	46												
15.4																					
16	0.1329639	0.0221606	6481994	46	0.1468144	0.0567867	0.1357340	7202216	1	0.3822714	0.0373961	0.0013850	0.2382271	4681440	4	0.0332409	9722991	69	0.0401662	0498614	96	0.2008310
16.2					0.0013850	0.0152354	5706371	19													
16.3							0.0609418	2825484	76												
17	0.0277008	0.1274238	2271468	1	0.1385041	0.0069252	0.0470914	1274238	23	0.0747922	0.1855955	6786703	6	0.2105263	1578947	4	0.0013850	4155124	65	0.0623268	6980604	2	0.2839335
17.1																					
17.2						0.0013850	4155124	654													
17.3		0.0207562	3268698	1			0.1329639	8891968	8									0.0013850	4155124	654	
18	0.0013850	0.1717451	5235457	1	0.0775623	2686980	6	0.0055401	6620498	61	0.0055401	0.0734072	0221606	65	0.1509695	2908587	3	0.0249307	0.0720216	066482		0.2022160
18.1																					
18.2						0.0013850	4155124	654													
18.3		0.0249307	4792243	77				0.0498614	9584487	53								0.0498614	0.0720221	606648	2	0.0013850	4155124	654	
19	0.0027700	0.1246537	3961218	8	0.0401662	0498614	96				0.1204986	1495844	9	0.0166204	9861495	84						0.1038781	
19.1																					
19.2																		0.0041551	2465373	961	

19.3			0.00415512465373961					0.0152354570637119													
19.4																					
20			0.110803324099723			0.018005540166205					0.156509695290859		0.00138504155124654				0.1232686	0.0581717451523546			0.0069252
20.1																					
20.2																		0.00969529085872576			
20.3																					
21			0.128808864265928			0.00969529085872576					0.0373961218836565						0.1786703	0.0249307479224377			0.0013850
21.2																	0.0055401	0.0235457063711911			
21.3																					
22			0.0955678670360111			0.00692520775623269					0.0346260387811634						0.2049861	0.0138504155124654			
22.2																	0.0124653	0.0373961218836565			
22.3																					
23			0.0692520775623269								0.105263157894737						0.1523545	0.00277008310249307			
23.2																	0.0027700	0.03601108033241			
23.3																					
24			0.0470914127423823								0.114958448753463						0.1343490	0.00138504155124654			
24.2																	0.0013850	0.0221606648199446			
24.3																					
25			0.0166204986149584								0.102493074792244						0.0789473684210526				
25.2									0.00138504155124654									0.0415512465373961			
26			0.00277008310249307								0.0304709141274238						0.0263157894736842				
26.2																		0.0415512465373961			
27			0.00138504155124654						0.0221606648199446								0.00415512465373961				
27.2																		0.0941828254847645			
27.3																					
28									0.159279778393352												
28.2																		0.0761772853185596			
28.3																		0.00138504155124654			
29									0.202216066481994												
29.2									0.00277008310249307									0.0554016620498615			
29.3																					
30									0.282548476454294												
30.2									0.0290858725761773									0.056786703601108			
30.3																					
31									0.07202216066482									0.00277008310249307			
31.2									0.0983379501385042									0.0235457063711911			
32									0.00554016620498615									0.00138504155124654			
32.2									0.0900277008310249									0.0124653739612188			
33									0.00138504155124654									0.00138504155124654			
33.1																					
33.2									0.0263157894736842									0.00415512465373961			
34																		0.00831024930747922			
34.2									0.00415512465373961									0.00138504155124654			
35									0.00138504155124654												
36									0.00138504155124654												

Appendix II

Table 2. Likelihood Ratios (LRs) computed for mixtures with estimated two contributors, on continuous software Euroformix.

Sample	LR
1	17,52427834
2	22,07846306
3	13,92057245
4	18,71250185
5	20,33566917
6	26,35188608
7	25,4177235
8	14,77684322
9	16,41363702
10	34,299881
11	16,52912347
12	28,1703727
13	23,42284945
14	8,253168861
15	17,61487587
16	20,65869146
17	18,59408431
18	18,58041984
19	25,39909522
20	16,7962994
21	22,94544918
22	13,6492157
23	16,92204362
24	20,18129605
25	22,13113004
26	26,200743
27	28,6344377
28	21,92153263
29	28,55648029
30	23,73273875
31	15,66453431
32	16,5812181
33	19,52471439
34	26,39864979
35	16,13322353
36	24,17578241
37	21,00795795
38	19,00185636
39	8,146391594
40	25,58652466
41	12,16563672
42	19,6882931
43	14,26198814
44	20,18675996
45	21,83100052
46	26,56983258
47	28,67936856
48	16,29791188
49	25,96950339
50	21,6599193
51	14,67224494
52	16,57730777
53	14,02413043
54	11,16804158
55	15,75524774
56	29,68202024
57	16,7768754
58	22,45720068
59	29,62974849
60	27,43708001
61	28,35911086
62	27,91804092
63	20,42177959
64	19,67533756
65	27,9851306
66	23,15788628
67	19,51739422
68	24,5152984
69	15,93085026
70	10,2187183
71	24,53918654
72	19,05519975
73	23,19906189
74	6,910568513
75	14,46263353
76	15,72497452
77	26,66957291
78	18,7661783
79	15,67174157

Table 3. LRs computed for mixtures with estimated two contributors, on qualitative software LRmix Studio.

Sample	LR
1	13,59221574
2	18,31238353
3	13,28694816
4	14,12759911
5	13,34072662
6	15,85271176
7	11,33990059
8	9,773291574
9	8,459968032
10	20,83194037
11	13,67777705
12	14,31944256
13	14,03490926
14	7,405694248
15	15,82187459
16	17,30965826
17	14,31079431
18	13,67932149
19	16,11687494
20	13,59442284
21	14,83147324
22	9,497759924
23	11,17615386
24	18,90347567
25	18,5064023
26	12,29031544
27	20,2975521
28	12,35857733
29	15,41872449
30	15,79539352
31	13,78060353
32	14,01536345
33	13,48830641
34	12,24759506
35	13,87206516
36	13,43063649
37	17,38650941
38	15,97787018
39	5,680035923
40	16,04126649
41	12,12070944
42	14,78408289
43	10,58183388
44	16,50789866
45	14,86010405
46	13,5960462
47	14,18357385
48	8,935058211
49	13,02048264
50	11,50469608
51	11,43004412
52	14,71940267
53	13,92918693
54	9,472826685
55	18,00197589
56	10,71202844
57	14,62467145
58	15,5509191
59	14,69958953
60	16,76212636
61	14,00102049
62	15,64780668
63	15,79579188
64	15,47873218
65	14,0630082
66	12,98362489
67	14,21535825
68	16,82552327
69	10,98514367
70	7,847115911
71	15,38172788
72	12,16805597
73	13,34261721
74	3,261555867
75	15,09533013
76	14,04934493
77	11,1455232
78	15,36059397
79	14,01800874

Table 4. LRs computed for mixtures with estimated three contributors, on quantitative software Euroformix.

Sample	LR
1	25,96175217
2	10,30406077
3	16,74181879
4	17,29194202
5	16,73601387
6	20,16802033
7	16,17726457
8	13,10073723
9	11,16264278
10	12,39297162
11	20,05640249
12	7,241058246
13	12,11081109
14	16,61975881
15	27,25297532
16	24,60469335
17	22,07732646
18	14,31340131
19	26,04368452
20	26,78568969
21	19,24050988
22	11,28849832
23	23,89305339
24	21,61403252
25	22,63081351
26	24,41491526
27	6,358119931
28	23,6901017
29	23,43942414
30	9,513680112
31	6,192620689
32	10,7048677
33	10,74614236
34	13,95820546
35	7,865718477
36	18,79518059
37	7,027796576
38	13,87520095
39	22,84191017
40	10,28660231
41	9,45870285
42	14,57281901
43	23,12522708
44	17,72145184
45	14,22284846
46	20,42956791
47	24,59329948
48	15,96009148
49	17,51777341
50	22,54019594
51	19,41460441
52	7,253745082
53	17,56805915
54	18,76370051
55	12,14733056
56	22,02524259
57	29,54085159
58	12,62806528
59	5,745282118
60	23,68339424
61	18,64515387
62	12,02255708
63	15,24921495
64	9,049001109
65	10,54670444
66	22,40434486
67	10,42897583
68	12,22331024
69	11,94126957
70	10,93213593
71	12,14503135
72	10,69512214
73	23,29161059
74	27,1424319
75	24,90720826
76	26,49853566
77	25,11538388
78	18,8741898
79	13,13284016

Table 5. LRs computed for mixtures with estimated three contributors, on qualitative software LRmix Studio.

Sample	LR
1	13,87565618
2	10,51363435
3	11,01929646
4	10,73916889
5	9,981071879
6	10,34886604
7	11,22227038
8	8,800895848
9	10,7685534
10	8,311645463
11	8,389030981
12	6,742190102
13	7,909683986
14	8,215566604
15	7,085112279
16	11,8792249
17	10,51765646
18	8,307925484
19	8,239264557
20	13,76256574
21	12,36764573
22	6,587455982
23	7,933851292
24	8,850130801
25	10,86930593
26	14,59616282
27	4,213117518
28	11,0685932
29	11,51108429
30	4,519985359
31	5,765630315
32	7,826196286
33	10,32307602
34	10,37709153
35	3,340479337
36	10,86529213
37	2,184035297
38	7,913207124
39	8,207974526
40	8,59894201
41	8,132716561
42	12,0512641
43	10,00403356
44	8,956715454
45	9,701229254
46	14,4615856
47	9,76015136
48	9,348587082
49	7,091176493
50	9,489518764
51	10,86966487
52	4,918462889
53	9,106147738
54	10,32473584
55	9,619389292
56	12,17700358
57	11,68501548
58	9,114603424
59	5,408284995
60	7,525709486
61	12,17542886
62	9,00944683
63	8,995036532
64	8,460106397
65	9,388327568
66	10,24295877
67	5,885593318
68	9,63096008
69	10,54396315
70	7,544634737
71	9,007874429
72	8,330667718
73	9,245541328
74	9,333004998
75	8,981186252
76	12,7887345
77	14,19983799
78	9,487287588
79	7,883255165

Table 6. LRs computed for mixtures with estimated two contributors assuming three contributors, on quantitative software Euroformix.

Sample	LR
1	17,54478699
2	22,08666546
3	13,99299014
4	18,72266013
5	20,71813192
6	26,35297244
7	25,28230669
8	16,01273057
9	16,67948423
10	34,22317387
11	16,53306562
12	28,17148385
13	23,41804919
14	8,79714267
15	17,61487427
16	20,65872792
17	18,59418361
18	18,58042286
19	25,39309134
20	16,80828941
21	22,94723665
22	15,83359088
23	17,53489319
24	20,18049917
25	22,13532501
26	26,20247779
27	28,63443831
28	21,92203137
29	28,55526668
30	23,72542381
31	15,6643208
32	16,58184128
33	19,47184348
34	26,39936558
35	16,12742834
36	24,1824431
37	21,0079618
38	19,00185383
39	7,860231898
40	25,5897907
41	12,17063905
42	20,74497693
43	14,23530933
44	20,18834337
45	22,33691827
46	26,57007551
47	28,67941112
48	16,30088921
49	25,97006108
50	21,5723298
51	14,69088533
52	16,57652811
53	14,01402499
54	11,73509269
55	15,81709117
56	29,6824443
57	16,7884237
58	22,46943392
59	29,51811983
60	27,44758764
61	28,35720677
62	27,92036223
63	20,90403618
64	19,67131779
65	27,9740343
66	23,15733073
67	19,81628696
68	24,52314692
69	16,85724534
70	9,953917372
71	24,51428268
72	19,06068776
73	22,17386272
74	10,42708162
75	14,47109934
76	15,71986917
77	26,47159499
78	18,76699365
79	15,66898213

Table 7. LRs computed for mixtures with estimated two contributors assuming three contributors, on qualitative software LRmix Studio.

Sample	LR
1	13,33203419
2	16,41939273
3	12,07896164
4	12,4258036
5	11,68362059
6	14,14572947
7	10,07452843
8	8,622860869
9	8,287939711
10	18,31006267
11	12,27203258
12	13,13098245
13	13,33095722
14	8,183648463
15	14,1416433
16	15,66423087
17	12,65928702
18	12,36326013
19	14,21065247
20	11,87634969
21	13,33811449
22	11,48721597
23	10,61477025
24	16,79384235
25	17,28580782
26	11,24166608
27	17,80597956
28	12,19346642
29	13,95320169
30	13,96000284
31	12,31588492
32	12,67674183
33	12,40283316
34	11,36267772
35	12,14129118
36	12,08810157
37	15,38145253
38	14,53604402
39	5,396895759
40	14,29478426
41	11,49897886
42	13,85804469
43	10,13279981
44	15,19845822
45	15,01766806
46	12,40520474
47	12,79673814
48	8,510897379
49	12,35887589
50	10,69901937
51	10,41655485
52	13,19492087
53	13,30614363
54	11,16656658
55	15,91092306
56	9,981939207
57	13,73672558
58	14,0101257
59	13,26400273
60	14,89150049
61	13,04040213
62	14,23790885
63	14,33035012
64	14,07790754
65	12,07002993
66	11,39911854
67	14,54509547
68	15,15725003
69	11,95464633
70	7,880234589
71	13,21344108
72	10,78307487
73	11,28961843
74	5,974774233
75	13,6582288
76	12,9555363
77	10,49106467
78	13,64848762
79	12,73340525

Table 8. LRs computed for mixtures with estimated three contributors assuming two contributors, on quantitative software Euroformix.

Sample	LR
1	24,99478547
2	12,10485527
3	17,24969198
4	15,48441213
5	16,73543134
6	19,98504378
7	12,46164901
8	11,89785145
9	11,57416433
10	10,1948278
11	20,36455502
12	4,561902439
13	10,58233977
14	9,165056538
15	23,69193028
16	-73,61209925
17	21,46188977
18	4,672531011
19	23,68263443
20	27,15177490
21	16,26505674
22	11,42297853
23	23,41404972
24	13,25585714
25	22,66466217
26	24,57508732
27	5,466476012
28	23,49983373
29	23,09504476
30	6,772611772
31	-1,846343022
32	-17,70169119
33	3,20167438
34	15,48215197
35	0,465313572
36	17,60288028
37	7,418321737
38	12,27529398
39	22,85345159
40	10,04109536
41	9,002097764
42	15,30600463
43	22,90904345
44	11,69216861
45	13,4298034
46	20,64741577
47	24,97202441
48	13,90535794
49	9,250174243
50	22,36525514
51	17,57619902
52	3,834670992
53	17,43979262
54	19,24775559
55	11,78765649
56	20,97501423
57	29,76651201
58	11,40835976
59	5,435281096
60	24,13961233
61	18,98330599
62	11,29214769
63	14,59962542
64	-8,025670646
65	3,967519189
66	21,862799
67	8,664354968
68	12,23284889
69	11,94576545
70	5,169589589
71	8,40875373
72	9,880303969
73	22,49966132
74	27,23507792
75	22,18107626
76	26,47158977
77	23,54747908
78	15,85179985
79	11,34670854

Table 9. LRs computed for mixtures with estimated three contributors assuming four contributors, on quantitative software Euroformix.

Sample	LR
1	26,4977939
2	10,37970104
3	16,87298242
4	17,43652333
5	16,80413439
6	20,1401789
7	16,36475022
8	13,47017914
9	11,23220014
10	12,77470062
11	20,04991591
12	7,21804987
13	12,71242078
14	16,72141187
15	27,35264433
16	24,7478396
17	21,97224919
18	15,372589
19	25,65707951
20	26,58599309
21	19,68993230
22	11,01882234
23	23,34548515
24	21,23007357
25	22,62726313
26	24,39125005
27	6,382452391
28	23,68552149
29	23,42035361
30	9,572331298
31	6,423864374
32	10,70342681
33	10,85642922
34	13,96902227
35	8,432459392
36	18,62818371
37	7,977195007
38	15,2851179
39	22,66044939
40	10,25912937
41	9,532556281
42	14,69377517
43	23,12365056
44	17,70939458
45	14,26217872
46	20,41787056
47	24,58989054
48	15,75137997
49	17,78127416
50	22,14232571
51	19,60799956
52	7,574933809
53	17,9820954
54	18,7565207
55	12,64811732
56	22,73559375
57	29,52881169
58	12,74816786
59	5,450374398
60	23,68466893
61	18,6966981
62	12,13929366
63	15,0795726
64	8,735016791
65	10,276247
66	22,35090931
67	10,00891172
68	12,21695459
69	11,93736132
70	11,35977821
71	12,17072897
72	10,61673946
73	23,28340735
74	27,14235877
75	24,8780511
76	26,50926443
77	25,13900202
78	18,8661148
79	13,35499042

Table 10. LRs computed for mixtures with estimated three contributors assuming two contributors, on qualitative software LRmix Studio.

Sample	LR
1	14,38608388
2	11,39202022
3	12,32981919
4	10,55648327
5	10,51281853
6	9,862912926
7	11,72945484
8	8,897266365
9	11,64860919
10	5,713975596
11	9,486779917
12	5,164569911
13	5,579039358
14	8,443806045
15	3,846707849
16	10,20627375
17	9,820132146
18	3,325760872
19	8,02109238
20	14,93928301
21	13,16048694
22	5,233506511
23	7,060642523
24	8,522510649
25	11,90761418
26	16,73116999
27	2,369417752
28	11,27498516
29	12,82742493
30	0,327101314
31	4,383799725
32	4,413395709
33	11,50512713
34	11,32931733
35	-3,015395463
36	10,13785716
37	2,360575024
38	5,684040345
39	8,302888833
40	8,613630092
41	6,942981228
42	13,45046172
43	10,9315641
44	6,826214008
45	6,823073688
46	15,4019613
47	9,822704014
48	10,15828455
49	3,671278138
50	9,643532832
51	9,515658542
52	-1,568326751
53	9,701606552
54	11,62085758
55	9,243902147
56	13,10015045
57	12,83670305
58	7,815472402
59	4,436165382
60	8,425210955
61	13,32588072
62	8,282128443
63	8,347695675
64	7,17940646
65	10,12463795
66	9,258542385
67	4,328114548
68	9,902311343
69	11,51947563
70	2,585646659
71	6,10620856
72	6,184943231
73	9,996324644
74	8,495141352
75	6,885691289
76	13,17932977
77	16,15120397
78	7,356553633
79	5,686343183

Table 11. LRs computed for mixtures with estimated three contributors assuming four contributors, on qualitative software LRmix Studio.

Sample	LR
1	12,54263761
2	9,799746634
3	9,97882863
4	10,27771806
5	8,990019271
6	9,836746801
7	10,28870544
8	8,172681299
9	10,00391151
10	8,187088502
11	7,849929727
12	6,819685218
13	7,672711006
14	7,55795616
15	7,855885016
16	10,86432808
17	10,14715468
18	8,418961324
19	7,632548141
20	12,45123803
21	11,15242023
22	6,280580762
23	7,373118292
24	8,223001152
25	10,26521413
26	13,26909091
27	3,994603579
28	10,20001139
29	10,69142378
30	5,04647035
31	5,528199424
32	7,771903613
33	9,435180007
34	9,579138342
35	3,911363798
36	9,932860029
37	2,904577744
38	7,529949818
39	7,558819263
40	7,959548519
41	7,863345437
42	10,95668456
43	9,185535889
44	8,597092335
45	9,554888311
46	13,13116005
47	8,997584898
48	8,523840444
49	8,162096107
50	8,750316148
51	10,05887651
52	5,683606128
53	8,481179611
54	9,428753718
55	9,119377519
56	11,12329495
57	10,76859485
58	8,471609388
59	5,171219338
60	6,861704676
61	11,22028959
62	8,503984882
63	8,367927063
64	8,070640441
65	8,607014458
66	9,762222892
67	5,704664597
68	9,270978055
69	9,72423127
70	7,939713837
71	8,751240455
72	8,068055364
73	8,559015401
74	8,767973899
75	8,60578569
76	11,9934052
77	13,06661025
78	9,086469473
79	7,756489549

Table 12. LRs computed for mixtures with estimated two contributors with F_{ST}=0, on quantitative software Euroformix.

Sample	LR
1	20,34332418
2	25,98498734
3	15,01321282
4	20,52548145
5	21,1869196
6	27,56962988
7	26,3211669
8	15,42346918
9	17,25644136
10	39,62056608
11	17,67690994
12	29,48018867
13	25,82288025
14	9,399515484
15	19,90682285
16	23,86678908
17	20,09581062
18	19,96085321
19	26,81154775
20	17,65511523
21	25,06227326
22	15,40945428
23	18,41057874
24	23,96898039
25	27,94459143
26	27,4317983
27	32,472958
28	23,66773785
29	30,18481744
30	25,40852737
31	16,86403667
32	18,1115093
33	20,79915206
34	27,60652981
35	17,20378615
36	25,21176768
37	22,79686543
38	20,7082579
39	8,924835926
40	27,07499667
41	13,26658173
42	21,11678923
43	15,17202882
44	22,79825766
45	24,7653801
46	28,68570825
47	30,737002
48	17,44437309
49	27,51018412
50	22,76720515
51	15,70905644
52	17,88458834
53	16,24970276
54	12,26914997
55	16,60495865
56	31,84991017
57	19,82588234
58	24,29199119
59	31,39705224
60	30,16638257
61	31,1988818
62	30,30349381
63	22,6655062
64	20,89142769
65	29,23078528
66	24,07052818
67	21,68528737
68	26,60732819
69	17,11681223
70	11,19873927
71	26,59036984
72	19,96445418
73	24,18035224
74	8,453366562
75	15,78266853
76	17,45513567
77	27,75339926
78	20,64675998
79	16,85287366

Table 13. LRs computed for mixtures with estimated two contributors with F_{ST}=0.015, on quantitative software Euroformix.

Sample	LR
1	16,67996749
2	20,99017824
3	13,45015552
4	18,15363457
5	19,94696856
6	25,82859635
7	25,03451683
8	14,49506205
9	16,09522768
10	33,00604157
11	16,04271554
12	27,63529046
13	22,65800924
14	7,845342356
15	16,91237237
16	19,69143273
17	18,00487723
18	17,99545977
19	24,78395914
20	16,42447678
21	22,16732264
22	13,11990075
23	16,33078302
24	19,16991351
25	20,74194607
26	25,71238664
27	27,52236681
28	21,35262555
29	27,86787956
30	23,04269545
31	15,16194959
32	15,95038819
33	19,06573358
34	25,86903016
35	15,67588763
36	23,70929629
37	20,2894702
38	18,3151544
39	7,811580742
40	24,91202884
41	11,72889556
42	19,08569034
43	13,85541835
44	19,35508628
45	20,9363534
46	25,95364841
47	28,03260443
48	15,85508614
49	25,35294416
50	21,17712293
51	14,23271896
52	16,00519371
53	13,33300632
54	10,71263185
55	15,42057547
56	28,90211476
57	15,95023811
58	21,76223304
59	28,95167415
60	26,73566954
61	27,51617902
62	27,09428241
63	19,75276804
64	19,13198802
65	27,45900905
66	22,76184167
67	18,81931425
68	23,71835862
69	15,416117
70	9,809534895
71	23,90877187
72	18,67298101
73	22,77599319
74	6,477281708
75	13,91719688
76	15,13306364
77	26,24674342
78	18,10487267
79	15,16407109

Table 14. LRs computed for mixtures with estimated two contributors with F_{ST}=0.03, on quantitative software Euroformix.

Sample	LR
1	14,80937788
2	18,62499041
3	12,25148198
4	16,82152216
5	18,90061618
6	24,47900613
7	24,06283205
8	13,77252795
9	15,32517335
10	30,25312102
11	14,82263424
12	26,2921771
13	20,90119983
14	6,867912261
15	15,31115757
16	17,52724132
17	16,57020603
18	16,52706494
19	23,1823871
20	15,47078623
21	20,34624891
22	11,87983039
23	14,87920147
24	17,01143629
25	17,88983894
26	24,50092876
27	25,05716321
28	20,0359514
29	26,13029619
30	21,31005613
31	13,90522918
32	14,39820951
33	17,96623649
34	24,49651449
35	14,52334615
36	22,47477513
37	18,53028674
38	16,61664722
39	6,961623968
40	23,19127776
41	10,65664418
42	17,56026985
43	12,78791976
44	17,42717986
45	18,91388621
46	24,57246208
47	26,51493005
48	14,77727439
49	23,82840994
50	19,93246316
51	13,11319206
52	14,52913848
53	11,73554025
54	9,588054124
55	14,59415803
56	27,07382191
57	14,11604807
58	20,10688652
59	27,31680444
60	25,1954674
61	25,6543245
62	25,20737641
63	18,24996461
64	17,66327187
65	26,14053628
66	21,74633768
67	17,16320414
68	21,81562146
69	14,09417068
70	8,78559455
71	22,44838266
72	17,71596634
73	21,68650707
74	5,486137488
75	12,56315126
76	13,73399504
77	25,2058501
78	16,51683403
79	13,86332943

Table 25. LRs computed for mixtures with estimated two contributors with F_{ST}=0, on qualitative software LRmix Studio.

Sample	LR
1	16,04305185
2	21,89326204
3	14,3129602
4	15,86250593
5	14,09071444
6	16,99953691
7	12,11908024
8	10,56110864
9	9,468112442
10	26,10838918
11	14,73640544
12	15,49960152
13	16,65018008
14	8,589756715
15	17,99961658
16	20,40103328
17	15,53793044
18	14,71377413
19	17,36124404
20	14,46143986
21	16,53357527
22	11,26784618
23	12,37545874
24	22,62939205
25	23,83039915
26	13,36151747
27	23,53753497
28	14,09498511
29	16,74595497
30	17,12289196
31	14,80361968
32	15,27736801
33	14,87398251
34	13,10638173
35	15,03178589
36	14,25555791
37	18,82478748
38	17,33590003
39	6,556617849
40	17,4000765
41	13,24475749
42	16,04453663
43	11,21333666
44	19,01006839
45	17,57881764
46	15,66131789
47	16,17809381
48	10,17728428
49	14,38442286
50	12,43213405
51	12,31744596
52	15,87494255
53	16,07760748
54	10,58572761
55	12,17959998
56	20,08011437
57	17,49995259
58	17,03618157
59	16,3135484
60	20,18882678
61	16,64833024
62	17,85071696
63	18,11507848
64	16,67342478
65	15,04036418
66	13,85219262
67	16,45680453
68	18,61309971
69	12,00940404
70	8,793446623
71	17,33465428
72	13,07061079
73	14,19710698
74	4,615336356
75	16,44727249
76	15,69985468
77	12,12444819
78	17,04625604
79	15,07678706

Table 16. LRs computed for mixtures with estimated two contributors with F_{ST}=0.015, on qualitative software LRmix Studio.

Sample	LR
1	12,87860217
2	17,37596199
3	12,84469854
4	13,65173384
5	13,00376358
6	15,36310129
7	11,01205773
8	9,426479904
9	8,068295431
10	19,65144186
11	13,22279851
12	13,8419055
13	13,22937925
14	6,980632327
15	15,16268401
16	16,43319005
17	13,82577593
18	13,22820514
19	15,58775136
20	13,21893464
21	14,21597358
22	9,011139763
23	10,70314407
24	17,95116031
25	17,31091092
26	11,87027031
27	19,4017613
28	11,79182722
29	14,8605726
30	15,26072633
31	13,35072204
32	13,49398729
33	12,98019362
34	11,86510604
35	13,3741543
36	13,05852684
37	16,80820781
38	15,43382429
39	5,297857921
40	15,49358279
41	11,67360245
42	14,25757655
43	10,29349019
44	15,76833207
45	14,06127304
46	12,99627445
47	13,60622122
48	8,450273776
49	12,48582667
50	11,10351113
51	11,05711848
52	14,21312258
53	13,26967837
54	9,01133081
55	10,18195258
56	17,26309434
57	13,85479328
58	14,98201112
59	14,08958169
60	15,89480928
61	13,25120066
62	14,9033758
63	15,08678917
64	14,96501543
65	13,64508595
66	12,60528885
67	13,5341579
68	16,15253758
69	10,54132562
70	7,449539204
71	14,79196309
72	11,78824054
73	12,97761454
74	2,908873882
75	14,53656199
76	13,49264632
77	10,77080024
78	14,79308752
79	13,57031825

Table 37. LRs computed for mixtures with estimated two contributors with F_{ST}=0.03, on qualitative software LRmix Studio.

Sample	LR
1	11,29101453
2	15,34542211
3	11,71501448
4	12,53939392
5	12,11422725
6	14,11301831
7	10,18658939
8	8,526335148
9	7,107635973
10	17,21022844
11	12,06444227
12	12,65087115
13	11,37126779
14	5,956499645
15	13,66339282
16	14,49380688
17	12,62775875
18	12,06778508
19	14,24959512
20	12,254638
21	12,77756484
22	7,887667394
23	9,539455877
24	15,92227248
25	14,86463748
26	10,83322833
27	17,4006233
28	10,47724023
29	13,45608174
30	13,93368485
31	12,26835164
32	12,20516407
33	11,75162052
34	10,86113076
35	12,1105336
36	12,07164514
37	15,37795751
38	14,07824758
39	4,314794039
40	14,13623269
41	10,57406537
42	12,93558824
43	9,51942764
44	14,07474498
45	12,29881437
46	11,65480133
47	12,26393474
48	7,265128668
49	11,18271659
50	10,08068523
51	10,1129814
52	12,9038185
53	11,7506505
54	7,868831878
55	8,927824723
56	15,55200988
57	12,13590263
58	13,60202294
59	12,63595089
60	14,05791679
61	11,62184616
62	13,22197985
63	13,46629718
64	13,64844747
65	12,58543601
66	11,63104593
67	11,9265076
68	14,55009657
69	9,404015839
70	6,447877368
71	13,43578817
72	10,83669295
73	12,04711916
74	2,127908448
75	13,1493235
76	12,18856675
77	9,862221839
78	13,46252956
79	12,43255102

Table 48. LRs computed for mixtures with estimated three contributors with F_{ST}=0, on quantitative software Euroformix.

Sample	LR
1	29,14106686
2	12,4154631
3	17,92172804
4	19,22779105
5	18,42999247
6	21,88373907
7	17,62794434
8	14,56045436
9	12,97450197
10	13,20056688
11	20,71146683
12	7,990628403
13	13,57539993
14	17,4396585
15	29,41566881
16	27,5294924
17	23,96169154
18	16,08194012
19	27,1836166
20	29,86638669
21	21,56174311
22	12,09398249
23	24,91586586
24	22,60869758
25	23,69487619
26	26,6325873
27	6,947962492
28	25,73819318
29	24,98476424
30	10,44287825
31	7,053939821
32	12,04370631
33	12,10287937
34	16,28025774
35	8,558782919
36	20,54374919
37	7,425905543
38	15,42549221
39	23,73321425
40	11,58127779
41	10,25668703
42	15,87503191
43	24,63745541
44	19,71959721
45	15,79740529
46	24,29436353
47	26,09235106
48	17,16166486
49	19,28484064
50	24,44276135
51	20,8785098
52	8,953919759
53	18,49712143
54	20,05251
55	13,54841217
56	23,96945798
57	31,94064865
58	13,96015846
59	6,324910473
60	24,4487765
61	20,13719533
62	13,13199582
63	16,41013247
64	10,93043095
65	11,91653541
66	24,01342896
67	11,09241258
68	13,22119455
69	13,42537971
70	12,30918537
71	14,16374156
72	11,88186704
73	24,25954021
74	28,42592545
75	27,05234753
76	28,88532394
77	27,59679184
78	20,18691078
79	14,13658347

Table 19. LRs computed for mixtures with estimated three contributors with F_{ST}=0.015, on quantitative software Euroformix.

Sample	LR
1	24,97204817
2	9,726181333
3	16,22421045
4	16,57561528
5	16,12147987
6	19,47668253
7	15,63549384
8	12,57142858
9	10,52342333
10	12,02091942
11	19,74925288
12	6,938434548
13	11,6270057
14	16,28761703
15	26,60653074
16	23,90601522
17	21,30647228
18	13,67741974
19	25,56764254
20	25,80311249
21	18,52790496
22	10,94669343
23	23,44444485
24	21,20496528
25	22,18413593
26	23,6286283
27	6,094979181
28	22,84435775
29	22,80453505
30	9,136304545
31	5,893041704
32	10,15989781
33	10,24756781
34	13,30131265
35	7,556931583
36	18,12484786
37	6,862175309
38	13,44162229
39	22,44807399
40	9,786168028
41	9,106553377
42	13,98936
43	22,44222824
44	17,17666127
45	13,62973262
46	19,41721674
47	23,97066411
48	15,46587265
49	16,83546443
50	21,85848502
51	18,84910643
52	6,749535509
53	17,16550626
54	18,19776123
55	11,58206545
56	21,35279238
57	28,81040636
58	12,08238812
59	5,490502266
60	23,34110376
61	18,04204065
62	11,5483436
63	14,78850166
64	8,524401788
65	9,990525952
66	21,80721465
67	10,14604115
68	11,77549164
69	11,40302524
70	10,38657326
71	11,54162274
72	10,25450059
73	22,86552704
74	26,58690736
75	24,18330775
76	25,74088965
77	24,37521961
78	18,30440782
79	12,70263979

Table 20. LRs computed for mixtures with estimated three contributors with F_{ST}=0.03, on quantitative software Euroformix.

Sample	LR
1	22,71308371
2	8,475383819
3	14,89038649
4	14,92090844
5	14,67005763
6	17,73477883
7	14,30271473
8	11,31707484
9	9,047686882
10	11,01444779
11	18,90032273
12	6,199579867
13	10,50742753
14	15,48231035
15	25,13638084
16	22,48076351
17	19,40537189
18	12,23397931
19	24,39362243
20	23,56606374
21	16,88685115
22	10,09101114
23	22,29242912
24	20,2009131
25	21,05376174
26	21,82264093
27	5,396867064
28	20,74641376
29	21,1723585
30	8,216898472
31	5,206420961
32	8,842724546
33	9,102540943
34	11,86117373
35	6,74931973
36	16,53419244
37	6,457113467
38	10,92896649
39	21,4215285
40	8,591831654
41	8,193784814
42	12,50893782
43	20,58620574
44	15,94496645
45	12,21813872
46	17,2604719
47	22,42227341
48	14,26787844
49	15,21088712
50	20,2477793
51	17,46626785
52	5,608026905
53	16,14029945
54	16,74078749
55	10,20676736
56	19,78330023
57	27,13862811
58	10,73034355
59	4,835139039
60	22,44363288
61	16,53139978
62	10,35120004
63	13,64016578
64	7,380148657
65	8,702403589
66	20,37006895
67	9,432477248
68	10,61385922
69	10,12768636
70	9,08022621
71	10,17755133
72	9,214876703
73	21,7457764
74	25,14742595
75	22,41273624
76	23,98559928
77	22,71395657
78	16,84527885
79	11,61836043

Table 51. LRs computed for mixtures with estimated three contributors with F_{ST}=0, on qualitative software LRmix Studio.

Sample	LR
1	16,76866334
2	12,71004178
3	11,98542014
4	12,26455512
5	11,33666202
6	11,70834591
7	12,65522654
8	10,00076506
9	12,55708829
10	9,097497077
11	8,984259116
12	7,566916506
13	9,192225209
14	9,045455174
15	9,091999967
16	15,07091024
17	11,86009107
18	9,962380011
19	9,125821678
20	16,44276486
21	14,53610817
22	7,25336445
23	8,606741285
24	9,790635023
25	11,95783578
26	16,83241655
27	4,886491556
28	12,46487627
29	12,88202019
30	5,35259674
31	6,658418684
32	8,796902142
33	11,61536905
34	12,44461021
35	3,936516333
36	12,27060827
37	2,48966603
38	9,423468774
39	8,889043043
40	9,905986874
41	8,990657888
42	13,16257524
43	10,9904294
44	10,93731255
45	11,17744437
46	18,38970226
47	10,81850604
48	10,31582582
49	8,377961179
50	10,98742797
51	12,30899898
52	6,736085977
53	9,940238577
54	11,22327026
55	10,82572366
56	13,96443874
57	13,8550234
58	10,51178661
59	6,278721963
60	8,047432505
61	13,67430079
62	9,919227385
63	9,989026065
64	10,53950604
65	10,64710769
66	11,67664319
67	6,708882024
68	10,75636055
69	12,25136718
70	8,801701884
71	10,91161689
72	9,460026484
73	10,07327109
74	10,23106129
75	10,86135638
76	15,03727315
77	16,75807364
78	10,46756635
79	8,769449376

Table 62. LRs computed for mixtures with estimated three contributors with F_{ST}=0.015, on qualitative software LRmix Studio.

Sample	LR
1	13,06874662
2	9,948190119
3	10,60210957
4	10,16683234
5	9,515596072
6	9,836049845
7	10,70387085
8	8,385198645
9	10,14975269
10	7,961819814
11	8,124520678
12	6,408763945
13	7,504856232
14	7,878981931
15	6,559222865
16	11,10956285
17	9,976628303
18	7,718707381
19	7,874939567
20	12,96228743
21	11,72867701
22	6,307915197
23	7,64280077
24	8,46514044
25	10,41896768
26	13,81049724
27	3,916645867
28	10,54302242
29	10,98002771
30	4,187280306
31	5,450476643
32	7,424017778
33	9,843328757
34	9,821273941
35	3,078665536
36	10,33638562
37	2,060202777
38	7,500014258
39	7,915156301
40	8,095699917
41	7,757232076
42	11,57739694
43	9,583544436
44	8,431924195
45	9,154938187
46	13,52380573
47	9,328953171
48	8,947826904
49	6,612374979
50	9,005697744
51	10,33199365
52	4,410425277
53	8,747006322
54	9,937769244
55	9,143928732
56	11,57525818
57	11,05958845
58	8,54242858
59	5,019456746
60	7,29374405
61	11,58081997
62	8,620115115
63	8,616405442
64	7,899548126
65	8,874479895
66	9,72269162
67	5,532950119
68	9,141864621
69	9,920046361
70	7,053216952
71	8,454347799
72	7,915816237
73	8,892701918
74	8,945977178
75	8,465669914
76	12,12669856
77	13,43349642
78	9,071873577
79	7,508459556

.

Table 73. LRs computed for mixtures with estimated three contributors with F_{ST}=0.03, on qualitative software LRmix Studio

Sample	LR
1	11,3172831
2	8,72043697
3	9,54469175
4	8,82427368
5	8,45117668
6	8,6124686
7	9,47924258
8	7,4214007
9	8,75439228
10	7,05037457
11	7,4376816
12	5,59759776
13	6,59200872
14	7,06274473
15	5,42042732
16	9,50743751
17	8,65951563
18	6,39186315
19	6,99203153
20	11,2120359
21	10,3059118
22	5,61820107
23	6,90472682
24	7,52368156
25	9,30722333
26	12,0311688
27	3,15027285
28	9,290859
29	9,70564881
30	3,39060635
31	4,72067122
32	6,43673602
33	8,72992192
34	8,62170103
35	2,40468009
36	9,09931426
37	1,7674312
38	6,57884741
39	7,17510288
40	6,90230286
41	6,79444096
42	10,3859405
43	8,52765166
44	7,29758279
45	7,87755252
46	11,5877082
47	8,28059085
48	7,96893627
49	5,49577389
50	7,917138
51	9,06226059
52	3,23605661
53	7,83763811
54	8,95758293
55	8,00595596
56	10,1962617
57	9,6741939
58	7,13350817
59	4,00308844
60	6,69032013
61	10,1343003
62	7,63995649
63	7,70731578
64	6,67387209
65	7,63593966
66	8,4899317
67	4,6416783
68	7,89631447
69	8,44054242
70	5,89118123
71	7,22346466
72	6,94680996
73	8,00182058
74	7,96391527
75	7,30368776
76	10,6160513
77	11,7338596
78	8,03298617
79	6,57834739

Table 84. LRs computed for mixtures with estimated two contributors with drop-in=0, on quantitative software Euroformix.

Sample	LR
1	16,0220159
2	22,33665849
3	13,77838776
4	18,76695178
5	20,24741288
6	26,36221214
7	model couldn't explain the data
8	model couldn't explain the data
9	13,70884659
10	34,3484409
11	16,54712594
12	28,17893266
13	17,53039873
14	model couldn't explain the data
15	17,61483014
16	20,65893503
17	18,59439549
18	18,58042993
19	25,38441095
20	16,83720472
21	22,9521065
22	model couldn't explain the data
23	model couldn't explain the data
24	model couldn't explain the data
25	model couldn't explain the data
26	26,20856422
27	28,63444432
28	16,49597445
29	28,56066367
30	23,69842282
31	15,66356062
32	16,58392773
33	15,78956463
34	26,40548925
35	15,75675485
36	24,19720529
37	21,00796251
38	19,00185701
39	8,001166423
40	25,59568348
41	12,14817446
42	model couldn't explain the data
43	14,90172384
44	model couldn't explain the data
45	model couldn't explain the data
46	26,57065513
47	28,67955836
48	16,33227635
49	25,97859379
50	model couldn't explain the data
51	14,66601625
52	16,57365376
53	13,9546013
54	model couldn't explain the data
55	15,97049303
56	29,68748141
57	16,357507
58	22,48896906
59	29,64400043
60	model couldn't explain the data
61	model couldn't explain the data
62	27,93112898
63	18,1798774
64	19,67001058
65	model couldn't explain the data
66	model couldn't explain the data
67	model couldn't explain the data
68	24,55716949
69	model couldn't explain the data
70	model couldn't explain the data
71	24,54773749
72	19,07970984
73	23,36869909
74	model couldn't explain the data
75	14,49746869
76	15,70276101
77	model couldn't explain the data
78	18,76607156
79	15,66309461

Table 25. LRs computed for mixtures with estimated two contributors with drop-in=0.1, on quantitative software Euroformix.

Sample	LR
1	17,52811045
2	22,07453314
3	14,05675871
4	18,66806037
5	20,42315302
6	26,34180641
7	25,40549326
8	15,73503769
9	16,73298554
10	34,25596358
11	16,50934718
12	28,16187164
13	23,58335792
14	8,562938326
15	17,61492664
16	20,65842084
17	18,59373861
18	18,58040865
19	25,4161014
20	16,75165927
21	22,93866774
22	16,20794868
23	17,16862033
24	20,18129521
25	22,13113114
26	26,19256557
27	28,63443034
28	22,10801344
29	28,5503785
30	23,77132241
31	15,66560567
32	16,57821028
33	19,69354291
34	26,39170319
35	16,32934163
36	24,15553831
37	21,0079529
38	19,00185563
39	8,23727704
40	25,56443907
41	12,16945576
42	20,00998799
43	14,2563911
44	20,19633672
45	22,13568772
46	26,56891907
47	28,67915791
48	16,26175532
49	25,96055642
50	21,65989
51	14,67439219
52	16,57555676
53	14,08132323
54	11,55254278
55	15,52185587
56	29,67645333
57	17,07210131
58	22,42664968
59	29,61567902
60	27,43476598
61	28,35853604
62	27,90524653
63	20,82060122
64	19,68036158
65	27,98116721
66	23,2194069
67	19,95520255
68	24,48462449
69	16,46546864
70	10,29058314
71	24,52994904
72	19,02824025
73	23,00370647
74	7,7997495
75	14,42431713
76	15,74910759
77	26,66629439
78	18,7679183
79	15,67546455

Table 96. LRs computed for mixtures with estimated two contributors with drop-in=0, on qualitative software LRmix Studio.

Sample	LR
1	13,55466092
2	18,33830905
3	13,30835331
4	14,17807859
5	13,38543747
6	15,89576265
7	model couldn't explain the data
8	model couldn't explain the data
9	8,446284066
10	20,89429151
11	13,71817669
12	14,33798396
13	14,03295144
14	model couldn't explain the data
15	15,86090541
16	17,34939421
17	14,35282019
18	13,71215374
19	16,15885906
20	13,65523361
21	14,86997024
22	model couldn't explain the data
23	13,84886999
24	model couldn't explain the data
25	model couldn't explain the data
26	12,31745411
27	20,34090378
28	12,34506867
29	15,44146173
30	15,83995429
31	13,81841332
32	14,03239392
33	13,50207793
34	12,25928984
35	13,93476257
36	13,46572983
37	17,42263341
38	15,9998137
39	5,690897833
40	16,0829918
41	12,11402656
42	model couldn't explain the data
43	10,59581312
44	model couldn't explain the data
45	model couldn't explain the data
46	13,61917384
47	14,22352722
48	8,936973797
49	13,01067396
50	model couldn't explain the data
51	11,45885788
52	14,75685021
53	13,9148465
54	model couldn't explain the data
55	10,71325476
56	18,04326417
57	14,60346662
58	15,58189282
59	14,72905628
60	model couldn't explain the data
61	model couldn't explain the data
62	15,66450673
63	15,82665982
64	15,50296734
65	model couldn't explain the data
66	model couldn't explain the data
67	model couldn't explain the data
68	16,85674292
69	model couldn't explain the data
70	model couldn't explain the data
71	15,45176502
72	12,20822278
73	13,42088054
74	model couldn't explain the data
75	15,11998263
76	14,05969877
77	model couldn't explain the data
78	13,65931608
79	12,73971189

Table 107. LRs computed for mixtures with estimated two contributors with drop-in=0.1, on qualitative software LRmix Studio.

Sample	LR
1	13,63043903
2	18,28427074
3	13,26412327
4	14,07442531
5	13,29325845
6	15,80662562
7	11,29664118
8	9,740987615
9	8,473595473
10	20,76693183
11	13,63515706
12	14,29922527
13	14,03731497
14	7,698299601
15	15,78050002
16	17,26887053
17	14,26651658
18	13,645274
19	16,07238
20	13,53070194
21	14,79072087
22	13,22969103
23	13,82344683
24	18,86777713
25	18,51769558
26	12,26200584
27	20,25023276
28	12,3732525
29	15,39423734
30	15,74770193
31	13,74041255
32	13,9966403
33	13,47322682
34	12,23473527
35	13,80662551
36	13,39335431
37	17,34693306
38	15,95381642
39	5,669822036
40	15,9973406
41	12,12704108
42	15,023907
43	10,56810124
44	16,72952913
45	15,41030277
46	13,57174908
47	14,14194357
48	8,933054535
49	13,03016513
50	11,49318725
51	11,40030416
52	14,67936282
53	13,9436957
54	10,08101153
55	10,71028001
56	17,95767603
57	14,64598715
58	15,51769323
59	14,66862341
60	16,71605921
61	14,00652926
62	15,62946447
63	15,76329641
64	15,45277416
65	14,00081473
66	12,92895115
67	14,51039999
68	16,7922261
69	11,55944804
70	7,873552098
71	15,30802146
72	12,12563115
73	13,26109196
74	4,170291979
75	15,06811748
76	14,03783052
77	11,37378191
78	13,63651539
79	12,72638398

Table 118. LRs computed for mixtures with estimated three contributors with drop-in=0, on quantitative software Euroformix.

Sample	LR
1	model couldn't explain the data
2	10,26329208
3	16,71180138
4	16,2225108
5	16,78701261
6	20,18586871
7	16,07288249
8	13,01066843
9	11,08447276
10	12,2572821
11	20,06953748
12	7,144189799
13	12,1296008
14	11,22447568
15	model couldn't explain the data
16	17,89445994
17	22,13583922
18	12,89863824
19	26,12774596
20	26,88171451
21	19,18934851
22	11,27462957
23	23,97075415
24	21,65575947
25	22,62148155
26	24,33105972
27	6,466781769
28	23,68552989
29	23,43761201
30	9,432007086
31	5,917154094
32	10,70106855
33	10,3040798
34	14,01729234
35	7,789742336
36	18,86013243
37	model couldn't explain the data
38	12,34959554
39	22,9496782
40	10,24812843
41	9,373739745
42	14,52169955
43	23,1290443
44	17,7044368
45	14,2306298
46	20,44977672
47	24,61657543
48	15,9869835
49	model couldn't explain the data
50	22,60923885
51	19,34049034
52	7,148261737
53	13,49992601
54	18,75216086
55	11,9498932
56	19,07107004
57	29,55131626
58	12,5585752
59	5,767120947
60	23,69133003
61	18,51911774
62	11,65945806
63	15,31006945
64	8,988479056
65	10,51183096
66	22,40430296
67	10,44568443
68	12,19031425
69	11,90489058
70	10,81521062
71	12,06823718
72	10,62191389
73	23,27098259
74	27,14703251
75	24,91732107
76	26,52178068
77	25,20361018
78	18,90559135
79	13,00700536

Table 29. LRs computed for mixtures with estimated three contributors with drop-in=0.01, on quantitative software Euroformix.

Sample	LR
1	25,20397176
2	10,34623325
3	16,77141753
4	17,43042368
5	16,70770723
6	20,15270296
7	16,2750066
8	13,18303
9	11,22752214
10	12,51970525
11	20,04332969
12	7,322975622
13	12,09581337
14	16,96069935
15	27,33034741
16	24,78932046
17	22,02933697
18	14,50246249
19	25,96873009
20	26,69240111
21	19,28825283
22	11,30438787
23	23,82066947
24	21,57264723
25	22,64138535
26	24,50062543
27	6,249393068
28	23,69135124
29	23,44223823
30	9,587095181
31	6,395314457
32	10,70778372
33	10,9018864
34	13,91866308
35	7,945393979
36	18,74445828
37	7,239630795
38	14,28465751
39	22,74668114
40	10,32270207
41	9,527883413
42	14,62005168
43	23,12248149
44	17,74023991
45	14,21697309
46	20,41185342
47	24,56988687
48	15,93760361
49	17,69769723
50	22,47307995
51	19,48154288
52	7,34996759
53	17,79708349
54	18,77641571
55	12,33247346
56	22,36987085
57	29,53204927
58	12,69855004
59	5,722494278
60	23,67499168
61	18,75527189
62	12,1476958
63	15,21081845
64	9,094114829
65	10,59067886
66	22,40392889
67	10,40707141
68	12,25724638
69	11,96922796
70	11,0395075
71	12,21567037
72	10,76355284
73	23,31438668
74	27,13856888
75	24,88962238
76	26,47606261
77	25,01883284
78	18,84648013
79	13,23637005

Table 30. LRs computed for mixtures with estimated three contributors with drop-in=0, on qualitative software LRmix Studio.

Sample	LR
1	13,90790345
2	10,52306533
3	11,05093968
4	10,72726277
5	10,00537702
6	10,35772886
7	11,23908281
8	8,810504602
9	10,78101067
10	8,288826866
11	8,397580889
12	6,69515959
13	7,906760525
14	8,22460436
15	model couldn't explain the data
16	11,90091767
17	10,50345215
18	8,268921415
19	8,24512147
20	13,78343985
21	12,39670601
22	6,592394898
23	7,946365923
24	8,867949411
25	10,87951818
26	14,61643975
27	4,214404273
28	11,08818124
29	11,5247965
30	4,448700626
31	5,754870674
32	7,821394956
33	10,34369669
34	10,39013838
35	3,29835741
36	10,89108655
37	model couldn't explain the data
38	7,919275818
39	8,227165848
40	8,603086656
41	8,114550815
42	12,08804998
43	10,02260763
44	8,951666009
45	9,686280576
46	14,48233862
47	9,778182684
48	9,373969266
49	model couldn't explain the data
50	9,501340579
51	10,87349613
52	4,860796224
53	9,117699283
54	10,35237674
55	9,62285726
56	12,19212277
57	11,70069254
58	9,116415333
59	5,405911801
60	7,554269409
61	12,1953047
62	9,025754853
63	9,002450205
64	8,453293615
65	9,40341821
66	10,24933426
67	5,870905669
68	9,632221823
69	10,55616517
70	7,476242278
71	8,997831642
72	8,310308678
73	9,263673231
74	9,35169611
75	8,967584601
76	12,79509965
77	14,21474481
78	9,488611617
79	7,84735319

Table 31. LRs computed for mixtures with estimated three contributors with drop-in=0.01, on qualitative software LRmix Studio.

Sample	LR
1	13,84152448
2	10,50328526
3	10,98611475
4	10,74926204
5	9,954798606
6	10,33926549
7	11,20399685
8	8,790097574
9	10,75511728
10	8,333256124
11	8,379556645
12	6,78663298
13	7,912984036
14	8,205115122
15	7,379937492
16	11,85620621
17	10,53047212
18	8,346553922
19	8,230203165
20	13,73944277
21	12,33629224
22	6,582683899
23	7,920832891
24	8,831779079
25	10,8581725
26	14,57387455
27	4,211855694
28	11,04805543
29	11,49596571
30	4,58954361
31	5,775889561
32	7,832266848
33	10,30081995
34	10,36268393
35	3,385058892
36	10,83858024
37	2,467896171
38	7,907880671
39	8,188558873
40	8,594097798
41	8,149335376
42	12,01344453
43	9,983672115
44	8,96167317
45	9,716783356
46	14,43888626
47	9,740925657
48	9,32183629
49	7,405222867
50	9,476636534
51	10,86375045
52	4,978031366
53	9,092966654
54	10,29606395
55	9,615874423
56	12,15982115
57	11,66766063
58	9,112290768
59	5,410508514
60	7,496477197
61	12,15478797
62	8,992592784
63	8,986918289
64	8,466586315
65	9,371879347
66	10,23605556
67	5,899822699
68	9,629520071
69	10,53057484
70	7,611845874
71	9,018012512
72	8,349597033
73	9,22670628
74	9,313369793
75	8,993805446
76	12,78156212
77	14,18339975
78	9,485525988
79	7,916229793

Table 32. LRs computed for mixtures with estimated two contributors with λ=0.05, on quantitative software Euroformix.

Sample	LR
1	17,5254945
2	22,07757416
3	14,06428575
4	18,65254396
5	20,29825994
6	26,35425662
7	25,41812665
8	13,90606342
9	15,26429412
10	34,27939383
11	16,54702693
12	28,17102698
13	24,05274821
14	8,761951994
15	17,61483014
16	20,65893503
17	18,59439549
18	18,58042993
19	25,34396319
20	16,82281625
21	22,94758074
22	12,47876983
23	13,00896066
24	20,18129676
25	22,13112906
26	26,20036995
27	28,63444432
28	22,20186658
29	28,56580206
30	23,69986408
31	15,66360521
32	16,58385698
33	20,08118293
34	26,40381601
35	16,55915205
36	24,16637482
37	21,00796251
38	19,00185701
39	8,130340349
40	25,58004146
41	12,2904979
42	15,38308368
43	14,62441141
44	19,10672414
45	19,01847399
46	26,57065513
47	28,67955837
48	16,33221192
49	25,97857697
50	21,50420769
51	14,66831118
52	16,59803309
53	14,03348427
54	7,737733855
55	15,83878912
56	29,68395979
57	16,3596233
58	22,48882974
59	29,63376855
60	27,43921778
61	28,35964533
62	27,93104043
63	18,43438779
64	19,67792411
65	27,99314105
66	22,93517362
67	19,47679359
68	24,46333439
69	15,38607713
70	10,1326639
71	24,54773702
72	19,07970933
73	23,15292411
74	2,422990871
75	14,49220291
76	15,7065121
77	25,96052954
78	18,76729653
79	15,6621432

Table 33. LRs computed for mixtures with estimated three contributors with λ=0.05, on continuous software Euroformix.

Sample	LR
1	25,95101116
2	10,33008098
3	16,72489131
4	17,24937644
5	16,65765331
6	20,14574075
7	16,13669777
8	13,14274402
9	11,14708624
10	12,38837804
11	20,05088622
12	7,165197515
13	12,0355716
14	14,88978067
15	26,621719
16	25,00771354
17	22,07804722
18	14,13986438
19	26,06814155
20	26,81908361
21	19,21042138
22	11,26961033
23	23,95621393
24	21,6505975
25	22,61975282
26	24,31880454
27	6,274198387
28	23,66015225
29	23,43065296
30	9,479321828
31	6,2242047
32	10,70108305
33	10,30599377
34	14,01703533
35	7,842437358
36	18,76309691
37	4,937692079
38	13,75714611
39	22,83785792
40	10,27602177
41	9,434235972
42	14,55320333
43	23,11993544
44	17,67677356
45	14,22180808
46	20,43655301
47	24,57573437
48	15,9748281
49	17,37172869
50	22,45442196
51	19,49605185
52	7,287059689
53	17,57192121
54	18,67790489
55	12,00590203
56	22,06749204
57	29,53985196
58	12,6484272
59	5,795987112
60	23,68669231
61	18,69622235
62	11,95904122
63	15,21004905
64	8,965697764
65	10,61066103
66	22,41408836
67	10,44106612
68	12,15032363
69	11,98468447
70	10,94665209
71	12,16494668
72	10,67081446
73	23,28363738
74	27,13592286
75	24,90399893
76	26,50368877
77	25,11551612
78	18,89673496
79	13,13459711

Table 34. LRs computed for mixtures with estimated two contributors with threshold limit T=150, on quantitative software Euroformix.

Sample	LR
1	17,52887618
2	22,07177147
3	12,83121333
4	18,49356781
5	20,05135473
6	26,31967972
7	25,37521929
8	14,72566263
9	11,85076691
10	33,89820425
11	16,38509631
12	28,16354756
13	23,21769501
14	7,337316721
15	17,6182739
16	20,6369327
17	18,57982191
18	18,59167528
19	24,37133956
20	15,36718875
21	22,93326419
22	15,43984674
23	17,08446827
24	20,18195315
25	22,12915748
26	26,17246241
27	28,63447137
28	22,03084129
29	28,55980519
30	22,92311655
31	15,66024302
32	16,34580881
33	19,75082936
34	26,41875299
35	13,89192971
36	24,28187797
37	21,05768698
38	18,99483258
39	6,941971525
40	25,56053166
41	9,945443874
42	20,07991331
43	14,25748994
44	20,19435496
45	22,05012082
46	26,56279071
47	28,67948304
48	16,3344708
49	25,93496766
50	21,70960345
51	14,64722238
52	16,64556055
53	13,93211393
54	11,0949838
55	11,00057169
56	29,67543567
57	17,29365242
58	22,57358903
59	29,6090563
60	27,43326148
61	28,35783268
62	27,93659444
63	20,28513072
64	19,47387902
65	27,99185139
66	23,09697522
67	19,70326641
68	24,50865229
69	16,5283751
70	8,825227757
71	24,58048768
72	18,94451018
73	22,2045179
74	8,719352039
75	14,0682714
76	15,588538
77	26,61808864
78	18,59531233
79	15,49174541

Table 35. LRs computed for mixtures with estimated three contributors with threshold limit T=150, on quantitative software Euroformix.

Sample	LR
1	25,96829913
2	9,923733597
3	15,38363892
4	17,45195972
5	16,51908093
6	20,15049101
7	16,51233727
8	14,38264995
9	11,01429342
10	13,09684142
11	19,96500585
12	6,391317331
13	11,80850765
14	16,61883816
15	27,19195325
16	25,10626569
17	21,95369455
18	14,34719649
19	25,73117441
20	26,56786443
21	19,31020873
22	9,617311665
23	23,98710978
24	21,75055479
25	22,61060985
26	21,91264873
27	2,183102678
28	23,49363958
29	23,38445029
30	10,13564864
31	6,969899374
32	10,70345255
33	10,84239852
34	13,94174013
35	8,704997447
36	18,26542072
37	7,385519464
38	15,11385373
39	22,05633222
40	9,194840664
41	8,775212259
42	13,61465363
43	23,19050318
44	17,71695935
45	14,22142157
46	20,41136173
47	24,57962319
48	15,91257528
49	17,95329608
50	22,090888
51	21,6635693
52	9,583303734
53	15,67311566
54	18,60207049
55	12,49463606
56	21,84919616
57	29,53513407
58	10,68782169
59	5,172058594
60	23,62475385
61	19,23082811
62	10,64001936
63	15,20884493
64	8,519077564
65	9,66936825
66	22,29895422
67	7,268627196
68	11,74304197
69	11,77425251
70	11,39324672
71	12,54236598
72	10,855135
73	23,57907042
74	27,14006611
75	24,61912305
76	26,48378674
77	24,97789065
78	18,8969204
79	13,58785226

Appendix III

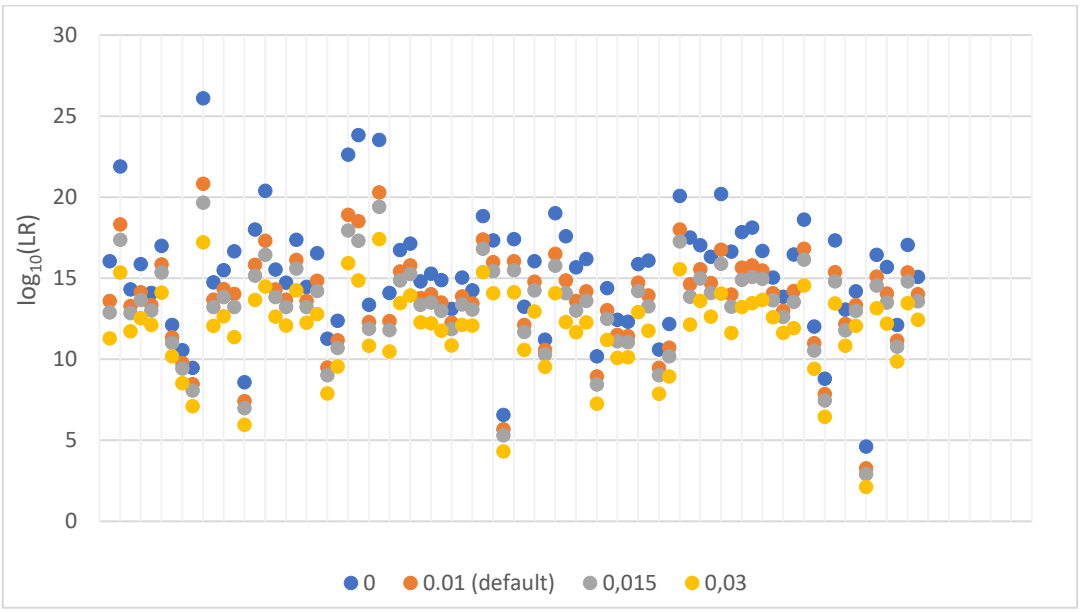


Figure 1. Plot showing the obtained LRs by LRmix Studio when the F_{ST} correction is varied in mixtures of two person estimated. Each vertical group of dots represents the LRs obtained for the same sample when F_{ST}=0 (blue dots), 0.01(default value; orange dots), 0.015 (grey dots) and 0.03 (yellow dots). Each set of four dots with the same x corresponds to the results obtained for a single sample (mixture/reference).

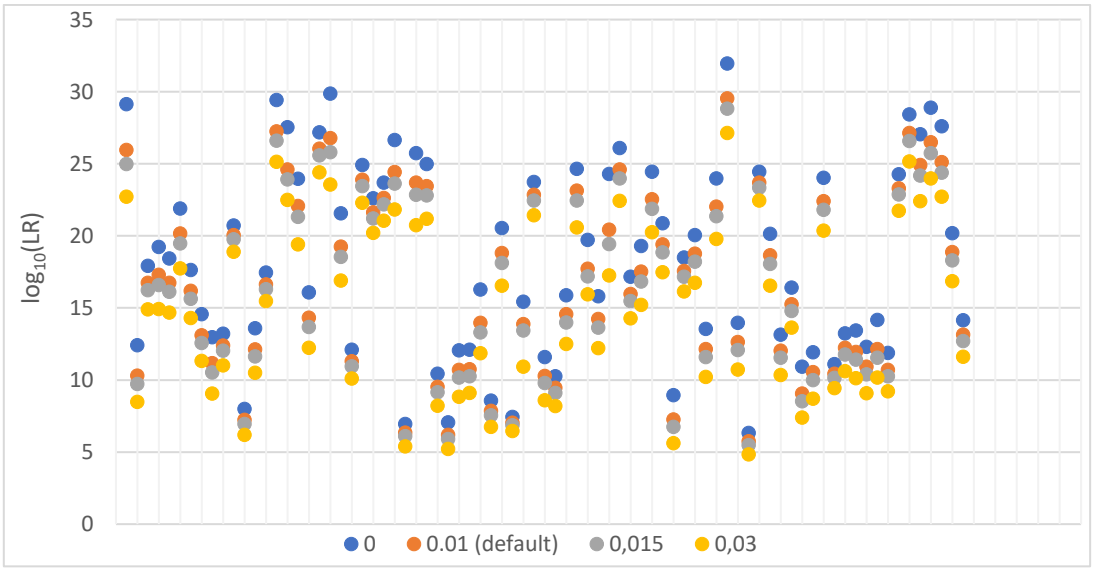


Figure 2. Plot showing the obtained LRs by Euroformix when the F_{ST} correction is varied in mixtures of three person estimated. Each vertical group of dots represents the LRs obtained for the same sample when F_{ST}=0 (blue dots), 0.01(default value; orange dots), 0.015 (grey dots) and 0.03 (yellow dots). Each set of four dots with the same x corresponds to the results obtained for a single sample (mixture/reference).

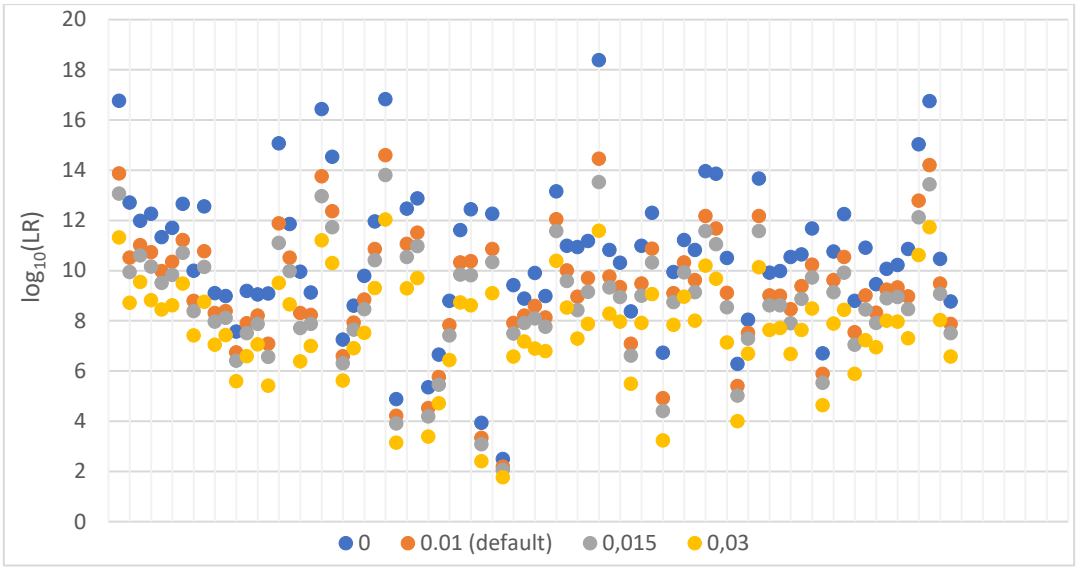


Figure 3. Plot showing the obtained LRs by Euroformix when the F_{ST} correction is varied in mixtures of three person estimated. Each vertical group of dots represents the LRs obtained for the same sample when F_{ST}=0 (blue dots), 0.01(default value; orange dots), 0.015 (grey dots) and 0.03 (yellow dots). Each set of four dots with the same x corresponds to the results obtained for a single sample (mixture/reference).

Appendix IV

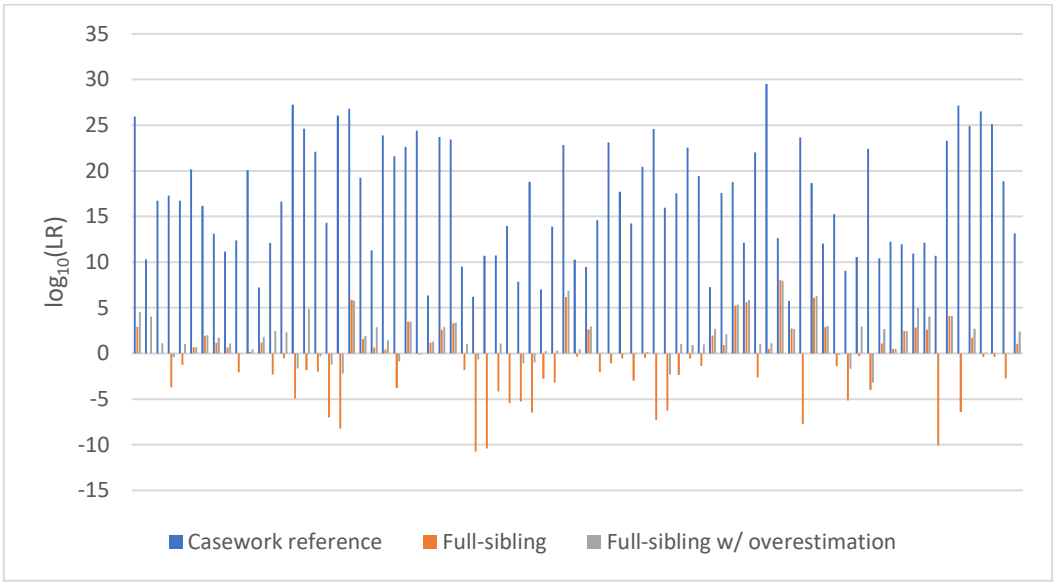


Figure 4. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated full-sibling (orange bar) and for a simulated full-sibling assuming four contributors (grey bar), in Euroformix.

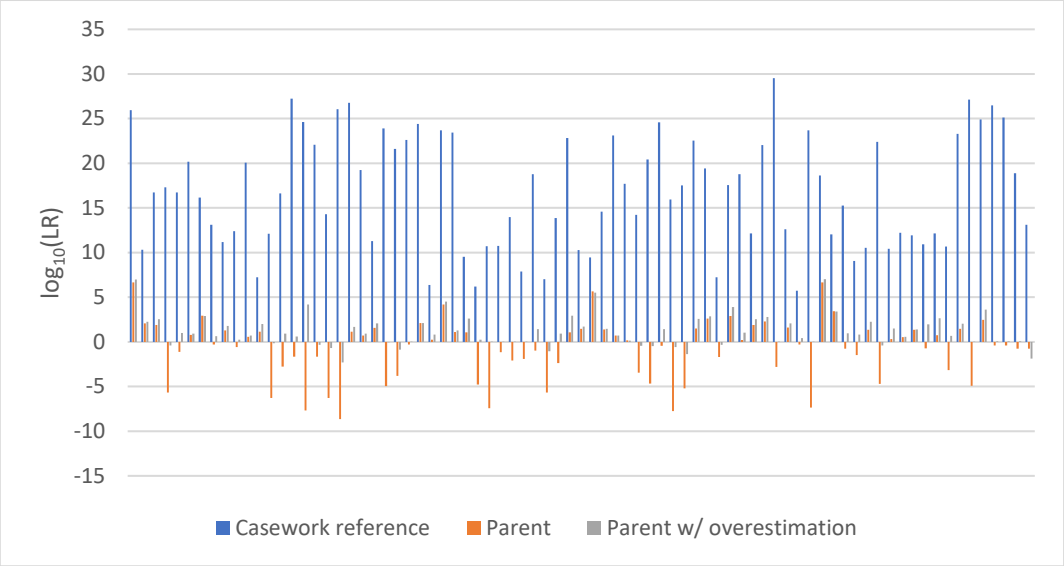


Figure 5. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated parent (orange bar) and for a simulated parent assuming four contributors (grey bar), in Euroformix.

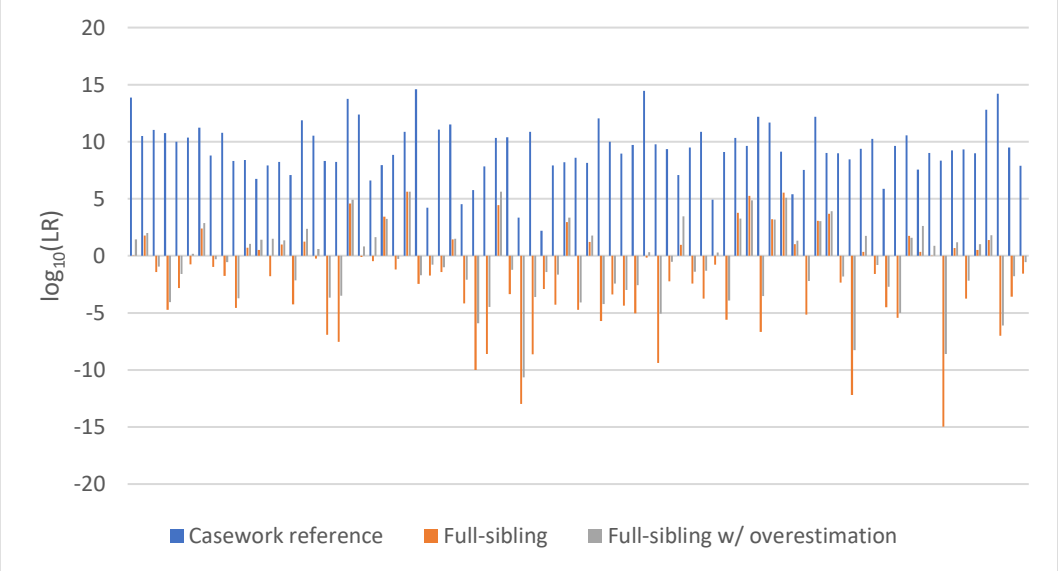


Figure 6. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated full-sibling (orange bar) and for a simulated full-sibling assuming four contributors (grey bar), in LRmix Studio.

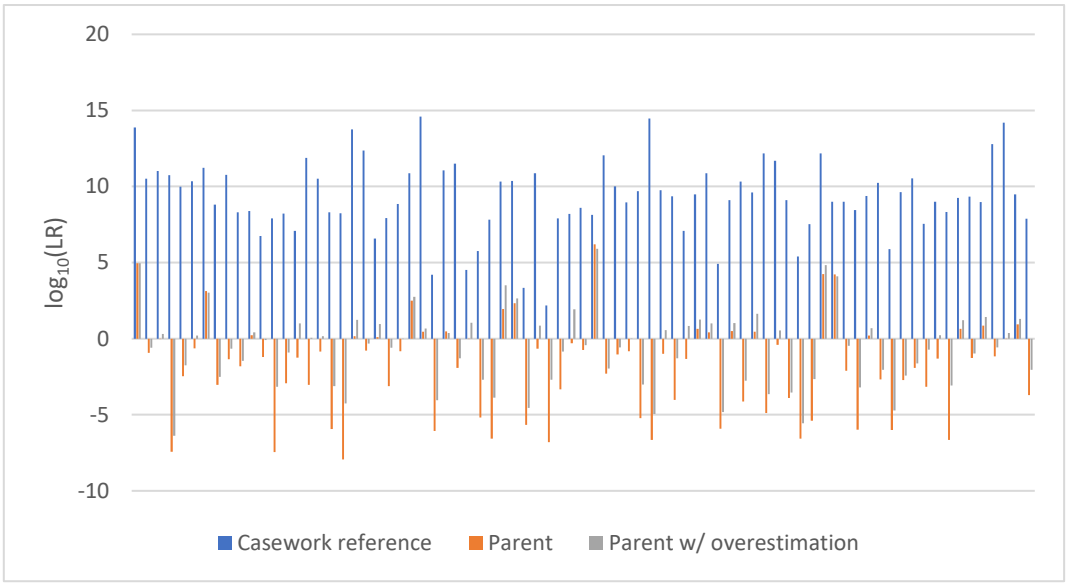


Figure 7. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated parent (orange bar) and for a simulated parent assuming four contributors (grey bar), in LRmix Studio.

Appendix V

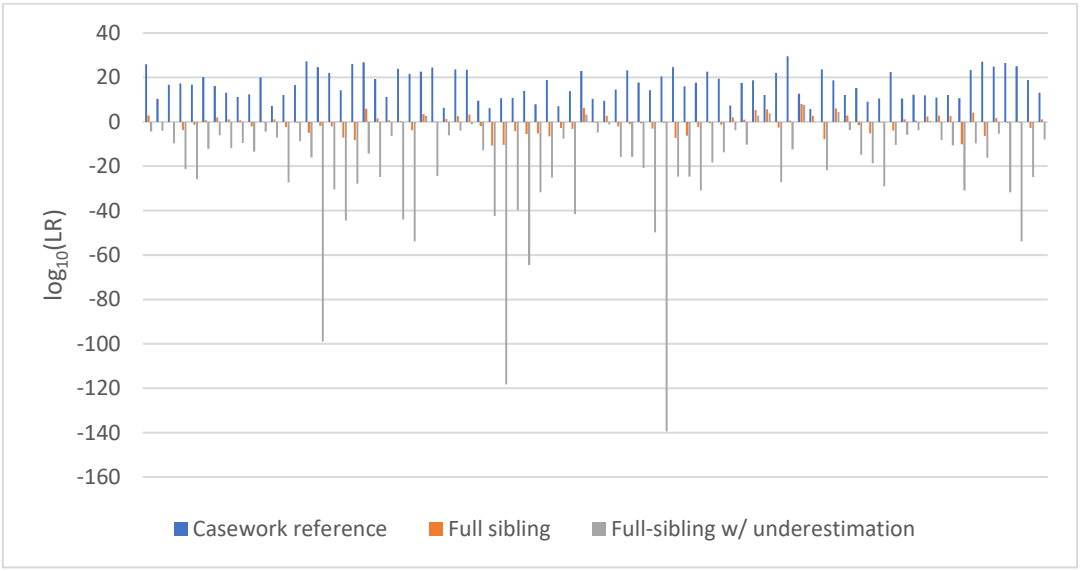


Figure 8. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated full-sibling (orange bar) and for a simulated full-sibling assuming two contributors (grey bar), in Euroformix.

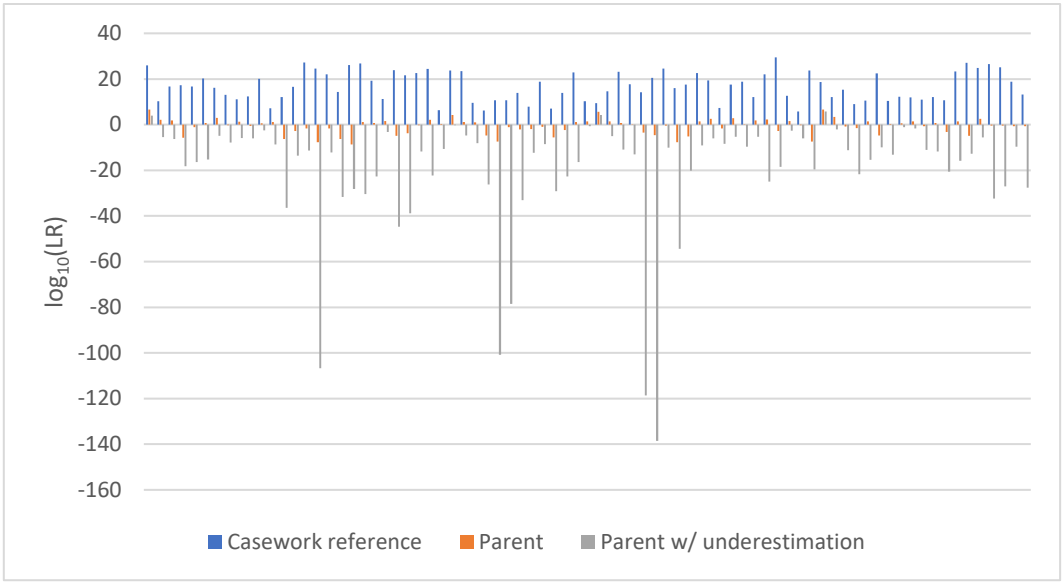


Figure 9. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated parent (orange bar) and for a simulated parent assuming two contributors (grey bar), in Euroformix.

Figure 10. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated full-sibling (orange bar) and for a simulated full-sibling assuming two contributors (grey bar), in LRmix Studio.

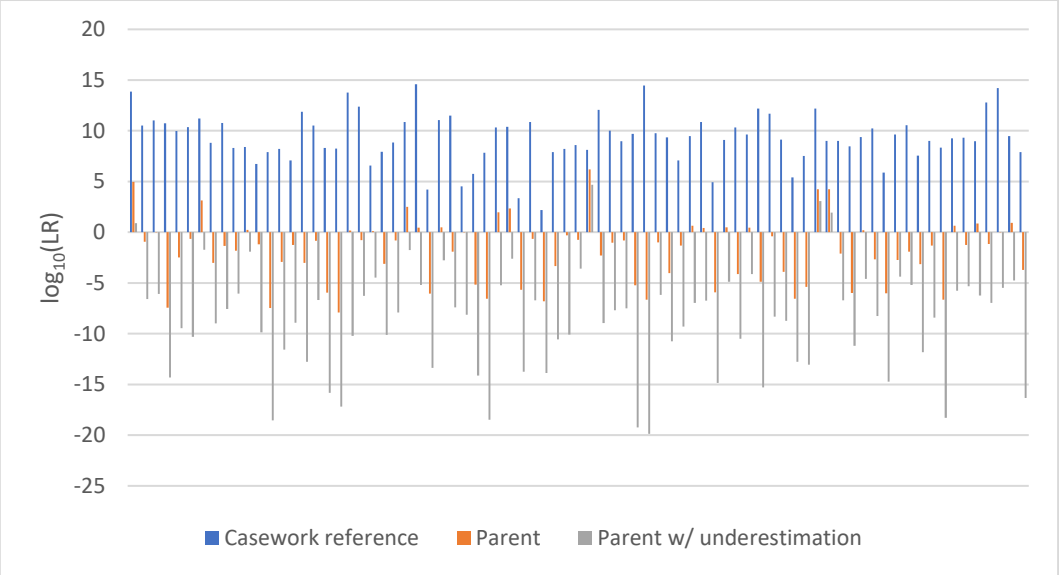


Figure 1. Computed LRs for each of the mixtures with three contributors estimated for the casework reference (blue bar), for a simulated parent (orange bar) and for a simulated parent assuming two contributors (grey bar), in LRmix Studio.