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Genetic and population analysis

Impact of allelic drop-out on evidential value of forensic DNA profiles using RMNE

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ABSTRACT

Motivation: Two methods are commonly used to report on evidence carried by forensic DNA profiles: the "Random Man Not Excluded" (RMNE) approach and the likelihood ratio (LR) approach. It is often claimed a major advantage of the LR method that drop-out can be assessed probabilistically.

Results: In this paper, a new RMNE measure is proposed that likewise accounts for allelic drop-out in an observed forensic DNA profile. We discuss the necessary calculations, underline their simplicity and provide a tool for performing the calculations.

Availability: An Excel file with preprogrammed calculations of RMNE probabilities for DNA profiles up to 16 loci and with a maximum of 2 drop-outs is available at:
http://www.labfbt.UGent.be/RMNE.php

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1 INTRODUCTION

There are two common methods to report on the probability of occurrence of forensic DNA profiles: the "Random Man Not Excluded" (RMNE) approach and the likelihood ratio (LR) approach. Each approach has its advantages and disadvantages. It is often claimed that the major advantage of the LR method rests in the ability to probabilistically assess the impact of drop-out (Gill *et al.*, 2006). For the RMNE we develop a method that is simple and allows for allelic drop-out while avoiding detailed probabilistic assumptions on where these drop-outs might have occurred. We discuss the necessary calculations and use them in some examples.

1.1 DNA profile and allelic drop-out

A DNA profile is a list of observed alleles from each of the analyzed loci. For each locus all possible alleles and their frequency of occurrence in the population are known. The alleles can be observed as an analog signal after PCR amplification of samples containing DNA from one or more individuals. It is possible that not all the alleles of the contributing individuals are observed. This allelic drop-out can result from various reasons: DNA degradation in the sample can lead to allelic drop-out, typically of the alleles with a longer product size. When very small amounts of DNA are present in the sample, stochastic effects can be the cause that the template DNA of some of the alleles is not sufficiently present in the PCR reaction. Due to technical imperfections of the used analysis methods, the signal of an amplified allele could fall below the signal-to-noise ratio threshold.

1.2 Random Man Not Excluded approach

In the forensic context the profile probability represents the probability $\Pr(E|H_0)$ of the evidentiary DNA profile (E) under the hypothesis (H_0) that the DNA profile is from a person unrelated to the suspect. For a single-contributor DNA profile, under the approximation that profiles from unrelated people are independent, this probability is the frequency of occurrence of the profile in the population (Gill *et al.*, 2006). This is the probability that a random person has the same DNA profile as the evidence profile; in other words that a random person is not excluded by the evidence.

The RMNE method doesn't make use of the quantitative data (peak height/area) in the DNA profile. Alleles are considered present only when observed and absent otherwise, ignoring the fact that there might be allelic drop-out. When there are loci that require dropped out alleles to allow for a match with the suspect sample, one practice is to omit the inconvenient locus from the RMNE calculation. Such a calculation is suspect-centric and prejudicial against the suspect as it implies that in the population considered by the calculation, only the same loci would be used for inculpation/exculpation as those being considered for the present suspect (Gill *et al.*, 2006). In this article however, we present a method using non suspect-driven RMNE calculations taking allelic drop-out into consideration.

1.3 The likelihood ratio approach

The LR approach considers the probability of the evidence under two or more alternative hypotheses about the source(s) of the profile. A typical analysis of a crime sample has the prosecution hypothesis (H_p) and the defense hypothesis (H_d).

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

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When the LR is less than one, the evidence favors H_d. When the LR is greater than 1, the evidence shows more support for H_p. In a single-contributor case, the LR coincides with the RMNE probability: The probability of the evidence profile under H_p (the suspect is the contributor) is 1, assuming that no errors are made in the chain of evidence. The probability of the evidence profile under H_d (the contributor is an unrelated person) is the population frequency of the profile as would have been given by the profile probability approach (Evett *et al.*, 1991; Gill *et al.*, 2006; Weir *et al.*, 1997).

The LR method also agrees on a binary view of alleles (see RMNE method). Published calculations (Gill *et al.*, 2000) and computer programs (Curran *et al.*, 2005) can be used to deal with allelic drop-out in a probabilistic way. The complexity of the LR calculation when mixed profiles and drop-out have to be considered is the reason why they are generally not used (Gill *et al.*, 2006). The biased practice of omitting the inconvenient locus from the LR calculation (as with the RMNE calculation, see above) is more commonly used.

1.4 Advantages and disadvantages of the RMNE and LR approach

The RMNE and LR approach are based on different statistical models which are elaborately discussed by Buckleton, 2005. The two methods are bound to give different answers in one and the same case (Gill *et al.*, 2006).

- In contrast with the LR approach, the RMNE approach does not make full use of the evidence. It does not make use of the information on the DNA profile of the suspect and the number of possible contributors (Buckleton, 2005), which may lead to an underestimation of the strength of the evidence.
- The RMNE approach is much more straightforward to implement, requires less interpretation (which is subject to non-objectivity) and returns a value which is valid for the mixed profile, independent of the knowledge of possible contributors. It can be reported before possible contributors have been identified and can be used as a quality "value" of the profile (Buckleton, 2005): When the RMNE frequency of an evidence profile is too high; this profile will show too many false positive matches with profiles of innocent suspects.
- The RMNE calculation is particularly useful in complex mixtures, because it involves no assumptions about the identity or number of contributors to a mixture (Buckleton, 2005). In the LR approach, the number of possible contributors has to be estimated (Gill *et al.*, 2006), which is subject to error.
- RMNE results are easier to explain in court: When a suspect matches the mixture, the main question is: "What is the probability that someone else in the population would also match the mixture?" (Buckleton, 2005). The RMNE calculation brings the exact answer to this question. LR results are more difficult to explain in court: Usually the defense hypothesis is not known, resulting in many LR options that might have to be discussed (Buckleton, 2005). Studies have demonstrated that there are serious problems with understanding evidence presented as a LR and that it takes more skill to correctly interpret the same evidence presented as a LR compared to presentation as a population frequency (Taroni and Aitken, 1998).
- Using the LR approach, the impact of drop-out can be assessed probabilistically (Gill *et al.*, 2006), but requires detailed assumptions on the drop-out mechanism and complex calculations. In this study however, we present an RMNE calculation which allows for allelic drop-out without being suspect-driven. The LR calculations for mixed contributor profiles assume a certain probability of the drop-out pattern P(D) (Gill *et al.*, 2006). This P(D) probability has to be estimated and is prone to error. One practice is to calculate LR for several P(D) (Gill *et al.*, 2006). To use the presented RMNE calculations, no assumptions have to be made about the probability of drop-out. The calculations simply allow for one, two or more drop-outs when comparing profiles of suspects and "random men" with the evidence profile.

2 METHODS

2.1 Single contributor, unambiguous profile

$P(E_L) = P(A)^2$ in case of homozygosity at locus L.

$P(E_L) = 2P(A_i)P(A_j)$ in case of heterozygosity at locus L.

With:

- $P(E_L)$ is the profile probability at locus L.
- $P(A)$, $P(A_i)$, $P(A_j)$ are the probabilities of the observed alleles A, A_i and A_j at locus L.

$$P(E) = \prod_{k=1}^{\lambda} P(E_{Lk})$$

With:

- P(E) is the profile probability (or RMNE probability) considering all analyzed loci.
- $P(E_{Lk})$ is the profile probability at locus L_k.
- λ is the number of analyzed loci.

2.2 Mixed contributor, unambiguous profile

$$P(E_L) = \left(\sum_{i=1}^{n} P(A_i)\right)^2$$

With:

- $P(E_L)$ is the profile probability at locus L.
- $P(A_i)$ is the probability of the observed allele A_i at locus L.
- n is the number of observed alleles at locus L.

$$P(E) = \prod_{k=1}^{\lambda} P(E_{Lk})$$

With:

- P(E) is the RMNE probability considering all analyzed loci.
- $P(E_{Lk})$ is the RMNE probability at locus L_k.
- λ is the number of analyzed loci.

2.3 Mixed contributor, considering the possibility of allelic drop-out

The calculation of the RMNE probability considering the possibility of allelic drop-out is based on the above described "mixed contributor" RMNE probability. We define the RMNE probability allowing for x drop-outs, as the probability that a random man would not be excluded as a donor to the mixture, where we will exclude someone if and only if more

than x alleles in his DNA profile are not observed in the mixture. Specifically, we calculate the RMNE probability conditional on the fact that 0, 1 or 2 allelic drop-outs may have occurred on any of the loci. To this end, we start by calculating the probability of a match at a given locus, assuming 0, 1 or 2 allelic dropouts, respectively, have occurred at that locus.

Probabilities at locus L:

$$P(E_{L0}) = \left(\sum_{i=1}^{n} P(A_i)\right)^2$$

$$P(E_{L1}) = 2\left(\sum_{i=1}^{n} P(A_i)\right)\left(\sum_{x=1}^{m} P(A_x)\right)$$

$$P(E_{L2}) = \left(\sum_{x=1}^{m} P(A_x)\right)^2$$

With:

- $P(E_{L0})$ is the RMNE probability at locus L, allowing no allelic drop-outs at locus L. This is the same formula used for unambiguous mixed contributor calculation.
- $P(E_{L1})$ is the RMNE probability at locus L, assuming that exactly 1 allelic drop-out has occurred at locus L. Only "random men" with a combination of an observed and a non-observed allele are not excluded.
- $P(E_{L2})$ is the RMNE probability at locus L, assuming that exactly 2 allelic drop-outs have occurred at locus L. Only "random men" with a combination of non-observed alleles are not excluded.
- $P(A_i)$ is the probability of the observed allele A_i at locus L.
- n is the number of observed alleles at locus L.
- $P(A_x)$ is the probability of the non-observed allel A_x at locus L.
- m is the number of non-observed alleles at locus L.

Probabilities combining the results from all loci:

The RMNE probability of a DNA profile allowing for no allelic drop-out $P(E_0)$, is the product of the $P(E_{L0})$ from all individual loci (Buckleton, 2005).

To calculate the RMNE probability of a DNA profile assuming exactly 1 allelic drop-out $P(E_1)$, one has to consider that this drop-out can occur at each analyzed locus. When this drop-out occurs at a certain locus (locus i), no drop-outs can occur at the other loci. The RMNE probability of a DNA profile assuming exactly 1 drop-out at locus i, is the product of the $P(E_{L1i})$ and the $P(E_{L0})$ of the other loci. $P(E_1)$ is the sum of all possible $P(E_L)$ products with 1 drop-out at one of the loci.

Reasoning by means of analogies, for calculation of the RMNE probability of a DNA profile assuming exactly 2 allelic drop-outs $P(E_2)$, one has to consider the possibility of 2 drop-outs at 1 locus or 1 drop-out at 2 loci. When 2 drop-outs occur at a certain locus (locus i), no drop-out can occur at the other analyzed loci. The RMNE probability of a DNA profile assuming exactly 2 drop-outs at locus i, is the product of the $P(E_{L2i})$ and the $P(E_{L0})$ of the other loci. The RMNE probability of a DNA profile assuming 1 drop-out at locus i and 1 drop-out at locus j, is the product of the $P(E_{L1i})$, $P(E_{L1j})$ and the $P(E_{L0})$ of the other loci. Accepting a random man as not being excluded when he matches any of these drop-out patterns, $P(E_2)$ is the sum of all possible $P(E_L)$ products with 2 drop-outs at 1 locus or 1 drop-out at 2 loci.

$$P(E_0) = \prod_{k=1}^{\lambda} P(E_{L0k})$$

$$P(E_1) = \sum_{i=1}^{\lambda} P(E_{L1i})\left(\prod_{1\le k\le\lambda;k\neq i} P(E_{L0k})\right)$$

$$P(E_2) = \sum_{1\le i,j\le\lambda;i<j} P(E_{L1i})P(E_{L1j})\left(\prod_{1\le k\le\lambda,k\neq i,j} P(E_{L0k})\right) + \sum_{i=1}^{\lambda} P(E_{L2i})\left(\prod_{1\le k\le\lambda;k\neq i} P(E_{L0k})\right)$$

$$P(E_{0to1}) = P(E_0) + P(E_1)$$

$$P(E_{0to2}) = P(E_0) + P(E_1) + P(E_2)$$

With:

- $P(E_0)$ is the RMNE probability considering all analyzed loci, allowing no allelic drop-outs.
- $P(E_1)$ is the RMNE probability considering all analyzed loci, assuming exactly 1 allelic drop-out at one of the analyzed loci.
- $P(E_2)$ is the RMNE probability considering all analyzed loci, assuming exactly 2 allelic drop-outs at one of the analyzed loci or two times 1 allelic drop-out at two of the analyzed loci.
- $P(E_{0to1})$ is the RMNE probability considering all analyzed loci, allowing up to 1 allelic drop-out at one of the analyzed loci.
- $P(E_{0to2})$ is the RMNE probability considering all analyzed loci, allowing up to 2 allelic drop-outs at 1 or 2 of the analyzed loci.
- $P(E_{L0k})$ is the profile probability at locus L_k, allowing no allelic drop-outs at that locus.
- $P(E_{L1i})$, $P(E_{L1j})$ is the profile probability at locus L_i and L_j, assuming exactly 1 allelic drop-out at locus L_i, and 1 at locus L_j
- $P(E_{L2i})$ is the profile probability at locus L_i, assuming exactly 2 allelic drop-out at locus L_i.
- λ is the number of analyzed loci.

3 IMPLEMENTATION

Table 1. Example of a hypothetical DNA profile with 3 loci.

Three loci with all known alleles	Population frequency of the alleles P(A)	Is the allele observed in the evidence profile?
LOCUS 1		
allele 1	0.18	
allele 2	0.19	Yes
allele 3	0.20	Yes
allele 4	0.21	Yes
allele 5	0.22	
LOCUS 2		
allele 1	0.15	
allele 2	0.15	
allele 3	0.16	Yes
allele 4	0.17	Yes
allele 5	0.18	Yes
allele 6	0.19	
LOCUS 3		
allele 1	0.12	
allele 2	0.12	
allele 3	0.13	Yes
allele 4	0.14	Yes
allele 5	0.15	Yes
allele 6	0.16	
allele 7	0.18	

3.1 RMNE calculation per locus based on the data in table 1

$$P(E_{L10}) = \left(\sum_{i=1}^{n} P(A_i)\right)^2 = (0.19+0.20+0.21)^2 = 0.36$$

$$P(E_{L11}) = 2\times\left(\sum_{i=1}^{n} P(A_i)\right)\times\left(\sum_{x=1}^{m} P(A_x)\right)$$
$$= 2\times(0.19+0.20+0.21)\times(0.18+0.22) = 0.48$$

$$P(E_{L12}) = \left(\sum_{x=1}^{m} P(A_x)\right)^2 = (0.18+0.22)^2 = 0.16$$

$$P(E_{L20}) = \left(\sum_{i=1}^{n} P(A_i)\right)^2 = (0.16+0.17+0.18)^2 = 0.26$$

$$P(E_{L21}) = 2\times\left(\sum_{i=1}^{n} P(A_i)\right)\times\left(\sum_{x=1}^{m} P(A_x)\right)$$
$$= 2\times(0.16+0.17+0.18)\times(0.15+0.15+0.19) = 0.50$$

$$P(E_{L22}) = \left(\sum_{x=1}^{m} P(A_x)\right)^2 = (0.15+0.15+0.19)^2 = 0.24$$

$$P(E_{L30}) = \left(\sum_{i=1}^{n} P(A_i)\right)^2 = (0.13+0.14+0.15)^2 = 0.18$$

$$P(E_{L31}) = 2\times\left(\sum_{i=1}^{n} P(A_i)\right)\times\left(\sum_{x=1}^{m} P(A_x)\right)$$
$$= 2(0.13+0.14+0.15)(0.12+0.12+0.16+0.18)$$
$$= 0.49$$

$$P(E_{L32}) = \left(\sum_{x=1}^{m} P(A_x)\right)^2 = (0.12+0.12+0.16+0.18)^2 = 0.34$$

3.2 RMNE calculation combining the results from all loci based on the data in table 1

$$P(E_0) = \prod_{k=1}^{\lambda} P(E_{L0k}) = 0.36\times0.26\times0.18 = 0.0168$$

$$P(E_1) = \sum_{i=1}^{\lambda} P(E_{L1i})\left(\prod_{1\le k\le\lambda;k\ne i} P(E_{L0k})\right)$$
$$= (0.48\times0.26\times0.18)+(0.50\times0.36\times0.18)$$
$$+(0.49\times0.36\times0.26) = 0.1007$$

$$P(E_2) = \sum_{1\le i,j\le\lambda;i<j} P(E_{L1i})P(E_{L1j})\left(\prod_{1\le k\le\lambda,k\ne i,j} P(E_{L0k})\right)$$
$$+\sum_{i=1}^{\lambda} P(E_{L2i})\left(\prod_{1\le k\le\lambda;k\ne i} P(E_{L0k})\right)$$
$$= (0.48\times0.50\times0.18)+(0.48\times0.26\times0.49)$$
$$+(0.36\times0.50\times0.49)+(0.16\times0.26\times0.18)$$
$$+(0.24\times0.36\times0.18)+(0.34\times0.36\times0.26)$$
$$= 0.2474$$

$$P(E_{0to1}) = P(E_0)+P(E_1)$$
$$= 0.0168+0.1007 = 0.1175$$

$$P(E_{0to2}) = P(E_0)+P(E_1)+P(E_2)$$
$$= 0.0168+0.1007+0.2474 = 0.3649$$

Table 2. RMNE probabilities for the hypothetical profile (see Table 1)

	Probability
$P(E_0)$ No drop-out	0.0168
$P(E_1)$ Assuming exactly 1 drop-out	0.1007
$P(E_2)$ Assuming exactly 2 drop-outs	0.2474
$P(E_{0to1})$ Allowing 1 drop-out	0.1175
$P(E_{0to2})$ Allowing 2 drop-outs	0.3649
$P(E_{0,\text{ omitting locus 1}})$	0.0459
$P(E_{0,\text{ omitting locus 2}})$	0.0635
$P(E_{0,\text{ omitting locus 3}})$	0.0936

3.3 Validation of the formulae on simulated populations

Three populations were simulated using two arbitrary chosen allele frequency tables. One population contains the given frequencies of all 256 allele combinations using 2 loci with 4 alleles each. A second population contains the given frequencies of all 6400 possible allele combinations using 1 locus with 5 alleles and 2 loci with 4 alleles. A third population contains the given frequencies of all 6561 allele combinations using 4 loci with 3 alleles each. These populations represent the "random men" who are included or excluded by a given arbitrary chosen evidence profile. For each individual is checked whether the individual would be included/excluded by the evidence, assuming 0,1 or 2 drop-outs or allowing up to 1 or 2 drop-outs. The frequency of included individuals (RMNE) in the simulated population was compared to the calculated $P(E_0)$, $P(E_1)$, $P(E_2)$, $P(E_{0to1})$ and $P(E_{0to2})$ respectively and found to be equal. An Excel file with these validation tests on 2 simulated populations is available at http://www.labfbt.UGent.be/RMNE.php.

4 DISCUSSION

In 2006, the DNA commission of the International Society of Forensic Genetics made recommendations on the interpretation of mixed DNA profiles (Gill *et al.*, 2006). These recommendations are generally endorsed by many forensic genetic laboratories. One important recommendation which is still debated regards the use of the LR method rather than the RMNE method (Morling *et al.*, 2007). To our knowledge, this is the first report on forensic RMNE calculations allowing for allelic drop-out. The reported calculations present an alternative to the poor practice of omitting an inconvenient locus from the standard RMNE calculation when the DNA profile of a suspect does not completely fit into the DNA profile of the evidence. This insight could tip the pro/con balance in favor of the RMNE method: The biggest advantage of the LR method is the fact that more data is used for the calculation, sometimes resulting in a less conservative result. The LR approach is a general and coherent framework for interpreting evidence, which allows combination with other evidence. In return, more assumptions have to be made (number and origin of possible contributors), several alternative hypotheses must be formulated (as the prosecution and the defense both seek to maximize their respective probabilities) and more complex calculations have to be performed.

4.1 RMNE allowing for drop-out

One of the main strengths of the RMNE approach is the possibility to report the RMNE probability before possible contributors have been identified. This probability is a measure for the usefulness of the profile for comparison with a suspect's profile or comparison with profiles in a database. The $P(E_{0to1})$ and $P(E_{0to2})$ calculations can be used to calculate the RMNE probability allowing for one or two drop-outs, without using the profile of a suspect.

In contrast with the LR approach, the presented RMNE approach does not need assumptions on the probability P(D) that an allelic drop-out has occurred in the evidence profile. Because it is impossible to estimate P(D) perfectly, one practice is to calculate the LR for a range of different P(D). This makes explanation in court of the LR even more confusing: Gill *et al.*, 2006 show that using the same mixed contributor profile and the same suspect, the LR result can shift from "evidence in favor of the prosecutor hypothesis" to "evidence in favor of the defense hypothesis" when using a different P(D). Using only one P(D) in court gives the false impression that the expert can actually make an estimation of the P(D). The presented RMNE calculation is a simpler measure with only one simple answer to the question: "How many random men would match the evidence when we allow for up to x number of allelic drop-outs". Logically, the probability that a random person is not excluded by the evidence increases with the number of allowed allelic drop-outs (see Table 2). Allowing for 1 drop-out using $P(E_{0to1})$ is in this example (see Table 1 and 2) more conservative than omitting 1 of either loci from the standard RMNE calculation, but this can not be generalized. Allowing for up to 2 allelic drop-outs using the $P(E_{0to2})$ formula is always more conservative than omitting one of either loci from the standard RMNE calculation (see Table 2).

When considering allelic drop-out, the presented RMNE calculation is conceptually simpler than the LR calculation: Instead of estimating the probability of drop-out, the RMNE calculation makes the simpler assumption that a given number of alleles may have dropped out and then accepts any profile in the population that matches that constraint as `not excluded'. Therefore no further assumptions need to be made about the independence or (relative) probability of drop-out at several loci. The main advantage is that in court, the RMNE measure gives only one simple answer to the question: "How many random men would match the evidence when we allow x number of allelic drop-outs". No alternative hypotheses and alternative probabilities of drop-out P(D) have to be discussed. On the other hand this simplification could be seen as a disadvantage: The probability that a drop-out has occurred in the evidence profile is of crucial importance for the evidential value against the suspect. If it is for example zero, the suspect should be excluded as the donor. Because it is however impossible to estimate the P(D) accurately, this leads to many alternative hypotheses, possibly confusing a jury of laymen (see previous paragraph).

4.2 RMNE, number of drop-outs and evidential value

The presented calculations can be adapted to find the RMNE probability, allowing for more than 2 drop-outs. The formula $P(E_3)$ (see below) calculates the RMNE probability assuming exactly 3 allelic drop-outs at 2 or 3 of the analyzed loci. Analogous formulae can calculate the RMNE probability allowing for an unlimited number of drop-outs. For each given mixed DNA profile, the $P(E_{0tox})$ can be calculated.

The question arises as to which x to use in the $P(E_{0tox})$ calculation and which $P(E_{0tox})$ to report in court. Several ways of working can be suggested.

An expert could set a maximum number of allowed drop-outs based on his expertise with the used analysis method and based on the quality (e.g. signal intensity) of the evidence profile. This assessment of the number of allowed drop-outs is less prone to error compared to the assessment of the probability that drop-out has occurred (needed for an LR calculation): While assessing if it is sufficiently probable that drop-out has occurred and how many drop-outs can be allowed, the expert indirectly makes an assessment of the P(D), in spite of the fact that a correct P(D) cannot be calculated. However the expert does not need to put down a distinct figure of the P(D) because the P(D) itself is not used in the RMNE calculation.

With 16 analyzed loci, the Powerplex16 (Promega, Madison, USA) and the AmpFlSTR® Identifiler (Applied Biosystems, Foster City, USA) are currently the commercially available forensic DNA profiling kits with the highest number of analyzed loci. Using this kind of kits, an expert would probably allow only up to 2, maybe 3 drop-outs. When more loci are analyzed, the chance that the analysis fails at one or more loci increases. In this case the limit in number of allowed drop-outs could be adjusted dependent on the number of analyzed loci.

Another scenario, where the presented RMNE formulas could be used is in the case that the same evidence sample is analyzed more than once. When a low quality profile is obtained from an evidence sample and when there is still enough DNA of the sample available, the expert could decide to perform a second PCR and subsequent analysis on the same sample. When mutually comparing the 2 obtained DNA profiles of the same sample, e.g. 2 drop-outs could be detected at locus A in profile 1 and 1 drop-out could be detected at locus B in profile 2. Based on this information, the expert can conclude with high certainty (not absolute certainty, because unlikely artifacts like drop-in could have occurred in this example) that drop-out has occurred in both these analyses. When drop-out is detected at 2 loci, there is a great chance that drop-out also has occurred at another locus. Using the presented $P(E_{0to1})$ and $P(E_{0to2})$ formulas, the expert could allow up to 2 drop-outs. Note that in this scenario, the expert does not have to estimate the P(D), as he can allow drop-out based on the fact that drop-out has occurred at two loci in the performed analyses.

The presented method should only be used if an expert decides that drop-out has to be taken into account. This decision should be based on the quality of the evidence profile and on an assessment if it is sufficiently probable that drop-out has occurred. Allowing for drop-out where $P(D)\approx 0$, could lead to the false inculpation of a suspect.

The number of allowed drop-outs (x) in the RMNE calculation should not be determined by the number of lacking alleles in the evidence profile compared with the suspect's profile. This could be considered a suspect-driven way of working as the number of allowed drop-outs is decided upon based on the profile of the suspect.

$$P(E_3) = \sum_{1\le h,i,j\le\lambda;h<i<j} P(E_{L1h})P(E_{L1i})P(E_{L1j})\left(\prod_{1\le k\le\lambda,k\neq h,i,j} P(E_{L0k})\right) + \sum_{1\le i,j\le\lambda;i\neq j} P(E_{L2i})P(E_{L1j})\left(\prod_{1\le k\le\lambda,k\neq i,j} P(E_{L0k})\right)$$

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