



# Effect of multiple allelic drop-outs in forensic RMNE calculations



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## ABSTRACT

Technological advances such as massively parallel sequencing enable increasing amounts of genetic information to be obtained from increasingly challenging samples. Certainly on low template, degraded and multi-contributor samples, drop-outs will increase in number for many profiles simply by analyzing more loci, making it difficult to probabilistically assess how many drop-outs have occurred and at which loci they might have occurred. Previously we developed a Random Man Not Excluded (RMNE) method that can take into account allelic drop-out while avoiding detailed estimations of the probability that drop-outs have occurred, nor making assumptions about at which loci these drop-outs might have occurred. The number of alleles that have dropped out, does not need to be exactly known. Here we report a generic Python algorithm to calculate the RMNE probabilities for any given number of loci. The number of allowed drop-outs can be set between 0 and twice the number of analyzed loci. The source code has been made available on <https://github.com/fvnieuwe/rmne>. An online web-based RMNE calculation tool has been made available on <http://forensic.ugent.be/rmne>. The tool can calculate these RMNE probabilities from a custom list of probabilities of the observed and non-observed alleles from any given number of loci. Using this tool, we explored the effect of allowing allelic drop-outs on the evidential value of random forensic profiles with a varying number of loci. Our results give insight into how the number of allowed drop-outs affects the evidential value of a profile and how drop-out can be managed in the RMNE approach.

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## 1. Introduction

Numerous scientific improvements have advanced the field of forensic DNA analysis. Technological advances enable increasing amounts of genetic information to be obtained from increasingly challenging samples. Highly degraded samples and samples with only a few template copies are routinely analyzed. The number of Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) loci that are being analyzed is ever increasing. With the advent of forensic applications of massively parallel sequencing (MPS), simultaneous analysis of 200+ STR and SNP loci is being developed and might soon be commercially released. With an increased number of analyzed loci, data analysis also becomes increasingly challenging. When more loci are analyzed, the chance that the analysis fails at one or more loci increases. Certainly on low template, degraded and multi-contributor samples, drop-outs and drop-ins will increase in number for many profiles, making it nearly impossible to probabilistically assess how many drop-outs

have occurred and at which loci they might have occurred. When so many loci are being analyzed, nearly all profiles from lower template evidence samples, even single contributor profiles, might be partial.

Previously we developed a Random Man Not Excluded (RMNE) method that can allow allelic drop-out while avoiding error-prone [1] estimations of the probability that drop-outs have occurred. There is no need to make assumptions about at which loci these drop-outs might have occurred [2]. This method handles the possible occurrence of allelic drop-out within the RMNE framework, which takes away one of the major concerns raised against the RMNE approach, restricting its use to DNA profiles where the profiles are unambiguous [12]. Our method tries to overcome this limitation by extending the RMNE model using an approach which is more inclusive but at the same time sufficiently conservative. The RMNE method of presenting the evidential value of a forensic DNA profile, is also called Combined Probability of Inclusion (CPI) or Combined Probability of Exclusion (CPE = 1 – CPI). The presented RMNE is a simple measure that answers the question: “How many random men would match the evidence when up to  $x$  allelic drop-outs are allowed?”. Mentioning “up to” is important as it means that a correct estimation of the number of drop-outs is not needed.

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With the here proposed approach, the RMNE probability can be calculated allowing for a number of drop-outs up to two times the number of analyzed loci. Logically, the probability that a random person is not excluded by the evidence increases with the number of allowed allelic drop-outs. If the number of allowed allelic drop-outs is two times the number of analyzed loci, the RMNE probability reaches 1, leaving no evidential value. In this study, a generic Python algorithm was developed to calculate the RMNE probabilities for any given number of loci. The number of allowed drop-outs can be set between 0 and twice the number of analyzed loci. The source code has been made available on <https://github.com/fvnieuwe/rmne>. An online web-based RMNE calculation tool has been made available on <http://forensic.ugent.be/rmne>. The tool can calculate these RMNE probabilities from a custom list of probabilities of the observed and non-observed alleles from any given number of loci. It generates the RMNE probabilities for a given range of allowed drop-outs. A graph is generated showing the increase of the RMNE probability over the range of allowed drop-outs. Using this tool we explore the effect of allowing allelic drop-outs on the evidential value of random forensic profiles with a varying amount of loci. Our results give insight into how the number of allowed drop-outs affects the evidential values of a profile and how drop-out can be managed in the RMNE approach. To our knowledge, we are the first to describe and provide an open-source tool for calculation of RMNE probabilities for any given number of loci and allowing drop-outs up to the mathematical maximum (twice the number of analyzed loci). Similar software developments for calculating likelihood ratios on profiles with allelic drop-out probability, are more widespread [3–6].

### 1.1. Interpretation of a DNA profile in the RMNE approach

A DNA profile is a list of observed alleles from each of the PCR amplified loci. These loci are chosen across all chromosomes and the autosomal STRs which are used for RMNE calculations are thus considered independent from each other. Using capillary electrophoresis, the alleles can be observed as an analog fluorescent signal. Using MPS, allele sequences are analyzed into a digital signal [7,8]. The signal strengths are only semi-quantitative for the amount of input of the allele template in the sample and are subject to stochastic effects originating from DNA degradation, inhibitors in the sample, low amounts of template and technical imperfections of the used analysis methods. It is possible that not all the alleles of the contributing individuals are observed. This allelic drop-out can be the consequence of various reasons: a mutation in the primer binding site causing a failure in the amplification of the allele; Allele sizes outside of the normal calling range for a particular locus could go undetected; DNA degradation in the sample can lead to allelic drop-out, typically of the alleles with a longer product size [9–11]; Due to stochastic effects and technical imperfections of the used analysis methods, the signal of an amplified allele could fall below the signal-to-noise ratio threshold or a predefined stochastic threshold.

The RMNE calculation does not make use of the quantitative data (fluorescence intensity, number of sequence counts) in the DNA profile. No assumptions are made about the number of contributors to the profile and the profile is not de-convoluted into alleles stemming from individual contributors to the profile. Alleles are considered present only when observed above a certain threshold and absent otherwise. Alleles below the threshold may not be used to support an inclusion. The potential for allelic dropout raises the possibility of contributors having genotypes not encompassed by the observed alleles. It is however possible to calculate RMNE probabilities that allow for allelic drop-outs while avoiding detailed probabilistic assumptions on where and with what probability these drop-outs might have occurred [2]. This

method avoids the suspect-centric method of omitting loci that lack one or more alleles to include a suspect [12].

The RMNE method is a two-step consecutive process. First, it is determined whether the suspect is included or excluded by the evidence profile. Secondly, when the suspect is included, a statistic is calculated to determine the fraction of the population that would also not be excluded as a contributor to the evidence profile. Some information that might be used in the exclusion phase is not used in the calculation of the RMNE statistic. For example, information on peak heights or profiles of persons who can be safely assumed to be in the mixture, may be used in the exclusion phase. This information is not used in the calculation phase. The genotype of the suspect is always used in the exclusion phase and then not used in the calculation of the RMNE statistic.

### 1.2. Drop-out in RMNE versus other statistical approaches

The “likelihood ratio” approach (LR) and the RMNE approach are the 2 main methods in use for the interpretation of mixtures. Both methods are widely used throughout the world. Whether one or the other is better, has been the subject of several reviews and heated debate [2,12–14]. It is out of the scope of this paper to review these differences, advantages and disadvantages. We however summarize how allelic drop-out is managed differently in both methods. For both approaches, it is a common practice that loci lacking one or more alleles are omitted from the calculation. This suspect-centric method should not be used [12], instead one of the methods outlined below should be applied.

The RMNE approach can allow for a number of allelic drop-outs without making detailed estimations of the probability that drop-outs have occurred nor making assumptions about at which loci these drop-outs might have occurred [2]. The number of alleles that have dropped out, does not need to be exactly known. The calculation answers the question: “How many random men would match the evidence when up to  $x$  allelic drop-outs are allowed?”. This is in contradiction with reviews stating that the RMNE approach assumes that all alleles are present and that the RMNE approach cannot adjust for the possibility of drop-out except by leaving out certain loci from the analysis [12,13,15]. Because stochastic effects make it uncertain that all alleles are accounted for, it is sometimes concluded that statistics that cannot allow for drop-out, should not be used. Allowing for dropouts in an RMNE approach can be necessary to account for dropouts that are common in casework mixtures. The intention of allowing these drop-outs is to have a more inclusive RMNE value to account for potentially unobserved alleles and not to include suspects that would otherwise be excluded because some of their alleles are missing in the profile.

In the LR approach, the probability  $P(D)$  is assessed that an allelic drop-out  $D$  has occurred in the evidence profile. This probability can be modelled based on amount of input DNA, peak height/area, decline in signal intensity with increasing amplicon length in the profile, previously determined drop-out rates based on similar samples in which the same inhibitors can be expected, etc. This  $P(D)$  value is used as one of the factors in the LR calculation. Since it is impossible to determine the  $P(D)$  perfectly [1], one practice is to calculate the LR for a range of different  $P(D)$ . This can be confusing: Gill et al. 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 broad range of  $P(D)$  values. Using only one  $P(D)$  in court gives the false impression that the expert can actually make a correct estimation of the  $P(D)$  [12]. When the  $P(D)$  can be correctly assessed, the LR approach will give a better estimation of the evidential value of a profile compared to the RMNE approach. When the  $P(D)$  is modeled based

on objective, quantitative data, this has the additional advantage that the assumptions used to calculate  $P(D)$  are transparent.

### 1.3. How many drop-outs can be allowed?

The question arises when and how many drop-outs can be allowed. This question can be tackled using different approaches. The next paragraphs describe two such procedures:

1. 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, complementary profiles in multiple analyses of the same sample) of the evidence profile, without making use of the suspect profile. Assessing if drop-out might have occurred is necessary: Allowing for drop-out when the probability  $P(D)$  that drop-out has occurred nears 0 in reality, could lead to the false inculpation of a suspect. 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  $P(D)$ , but the expert does not need to put down a distinct figure of the  $P(D)$  because  $P(D)$  itself is not used in the RMNE calculation. There is also no need to determine at which loci the drop-outs have occurred. On low template, degraded and multi-contributor samples, with many analyzed loci (like in MPS applications), the exact number of occurred drop-outs cannot be determined with absolute certainty. For this reason the RMNE probability is calculated allowing up to a number of drop-outs and not for an exact number of drop-outs. With each additionally allowed drop-out, the evidential value of the profile decreases. When the drop-out assessment would lead to allowing more DO than there are in reality, this is safeguarded by a meaningful decrease in the evidential value of the profile (see also point 2). Nevertheless, an arbitrary threshold could be set for the maximum number of drop-outs that can be allowed, to avoid incorrect assessments of the number of drop-outs to allow. This threshold could be adjusted based on e.g., the number of analyzed loci: when more loci are analyzed, the chance that the analysis fails at one or more loci increases.
2. As mentioned above, assessing if drop-out might have occurred in an evidence profile is necessary because allowing for drop-out where  $P(D) \approx 0$  in reality, could lead to the false inculpation of a suspect. For example, a complete single contributor profile with high signal strength,  $P(D)$  is nearing 0, and thus drop-outs should be allowed very exceptionally. After making the assessment that drop-outs are likely to have occurred, it might however be reasonable to use the suspect's profile in the determination of the number of drop-outs that should be allowed and to be lenient towards the number of drop-outs that are allowed. With an increased number of allowed drop-outs, the evidential value of the evidence profile decreases. When 2 allelic drop-outs are allowed per analyzed locus, the RMNE value for that profile reaches 1 and the profile has thus no evidential value at all. So in the extreme example that a suspect lacks all alleles in a profile and the RMNE is calculated allowing for all these "drop-outs", this "included" suspect would not be inculpated by the evidence profile. In this way of managing possible drop-out, the RMNE is calculated as if the suspect is included, with the number of allowed "drop-outs" that is needed to make this inclusion. The RMNE could be stated as "if we include the suspect in the evidence profile, 1 in  $x$  number of random men would also be included in this evidence profile". Because there is no inclusion/exclusion step prior to the RMNE calculation in this approach, it is important to have a suitable RMNE threshold to filter meaningful, useful, non-random inclusions from inclusions with too high corresponding RMNE

values. This RMNE threshold based filter could be adjusted based on the circumstance in which the evidence profile is used: e.g., based on the number of suspects in a case, the size of the used database etc.

Determining the number of lacking alleles in the evidence profile based on a comparison with the suspect's profile (as outlined above in approach 2) is a suspect-driven procedure as the number of allowed drop-outs is decided based on the profile of the suspect. This practice is debatable: the presence and absence of alleles in an evidence profile is indeed completely unrelated to the profiles of suspects, but to assess if and how many drop-outs have occurred, a suspect's profile could be used as one of the factors in the assessment. 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 suspect-centric calculation is prejudicial against the suspect as it implies that in the population considered by the calculation, only the same loci would be used for inclusion/exclusion as those being considered for the present suspect [12]. Our method overcomes this by using an extended RMNE approach which is more inclusive but at the same time sufficiently conservative. In our calculation, it is not determined at which specific loci drop-outs are allowed. All the loci are used for the present suspect and the reference population. A random person from the population that (better) fits the evidence profile with less drop-outs in this evidence profile compared with the present suspect, is however also included. If this random person would have been considered as suspect, the number of allowed drop-outs would have been less, leading to a higher evidential value of the evidence profile. Another random person from the population that fits the evidence profile only when allowing more drop-outs, is not included. If this random person would have been considered as suspect, the number of allowed drop-outs should have been higher, leading to a lower evidential value of the evidence profile.

## 2. Methods

### 2.1. Calculation of allelic drop-outs

All calculations and visualizations were performed using Python 3.3 and are based on previously reported calculations [2]. The RMNE probability  $P(E_{0 \text{ to } r})$  of a profile allowing up to  $r$  DO, is the sum of the RMNE probabilities  $\sum_{i=0}^r P(E_i)$  assuming an exact number of DO ranging from 0 to  $r$ . To calculate the RMNE probability assuming an exact number of  $r$  DO in  $n$  loci, each possible combination of  $r$  DO in  $n$  loci needs to be considered.

In other words, when calculating the RMNE probability with  $r$  number of drop-outs (DO) in  $n$  number of loci, basically all possible unique combinations of  $r$  number of DO that could happen in  $n$  number of loci need to be considered. The RMNE probability of each of these combinations needs to be calculated and summed. The total number of allowed DO is maximum 2 times the number of loci as maximum 2 DO can be allowed per locus.

Two Python scripts were developed to achieve this. These scripts mainly differ in the way the possible unique combinations are generated, not in the actual calculation of the RMNE probability. A "combination" is here defined as a list of loci in which drop-out has occurred; combinations with 2 DO at the same locus have this locus 2 times in the list. One script generates all mathematical combinations of  $r$  loci chosen from a list containing each analyzed locus two times (e.g., locus1, locus1, locus2, locus2, ...), resulting in  $2n!/(2n-r)!r!$  mathematical combinations. This algorithm is fast, but generates duplicate combinations that need to be filtered afterwards. This step requires that all combinations are loaded into memory and thus the number of combinations is

limited by the amount of available memory. The Python code for this algorithm can be found in Supplementary file 1. Another Python script generates all mathematical “combinations with repetition” of  $r$  loci from a list containing 1 instance of each analyzed locus (e.g., locus1, locus2, locus3, ...), resulting in  $(n+r-1)!/(n-i)!r!$  mathematical combinations. This algorithm generates combinations with more than 2 instances of the same locus. These combinations need to be filtered. The advantage is that these combinations can be filtered one by one. The combinations do not need to be loaded into the memory all at once, leading to a moderate memory requirement that almost does not increase with increasing numbers of loci and allowed DO. The algorithm is however slow as the number of mathematical “combinations with repetition” increases dramatically with the number of loci and the number of assumed DO. The Python code for this algorithm can be found in Supplementary file 2. The provided code contains comments to explain the algorithms in detail. It can be used to calculate any possible number of DO from any number of loci with any number of observed and non-observed alleles.

On-line calculations are provided through <http://forensic.ugent.be/rmne>. This website runs a multiprocessing version of the fast script on an 8-core server. The website currently accepts between 1 and 200 loci. The maximum number of allowed DO that can be calculated on the website is restricted to calculations that do not exceed 5 minutes calculation time. The calculation time is estimated beforehand using the formula  $2n!/((2n-r)!r!)$  with  $n$  the number of loci and  $r$  the number of DO.

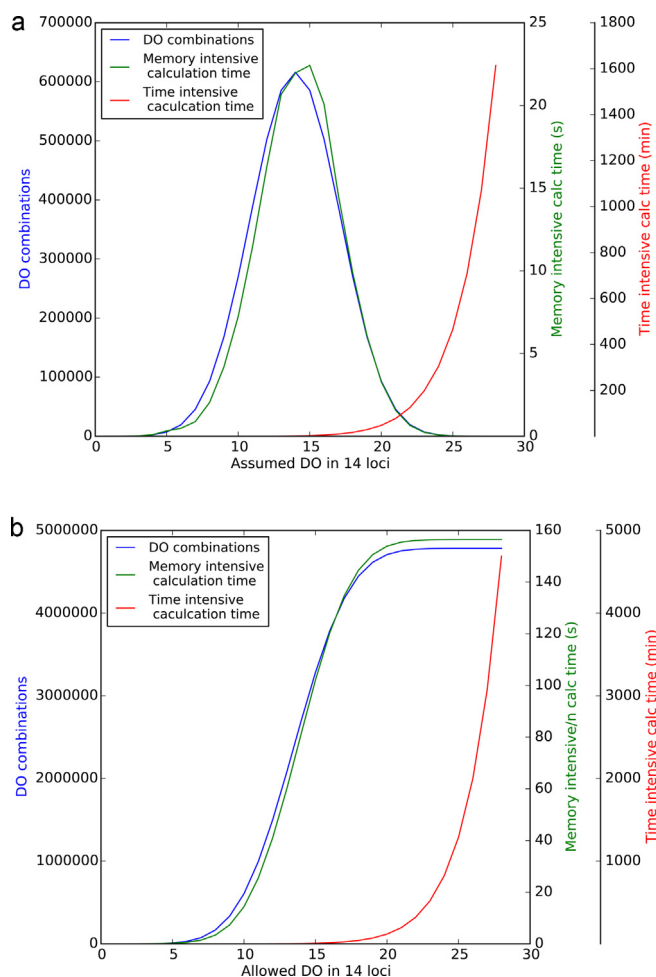
## 2.2. Generating random profiles

The random profiles were generated using Python 3.3. Allele frequencies were adopted from papers on the allele frequencies in the Belgian population [16,17]. Note that the minimum allele frequency is set to be 0.006. The use of a minimum allele frequency enables compensation for sparse sampling of infrequent alleles in population databases [18]. This also avoids that too much evidence value is inferred from the presence of a rare allele. A generic ‘exception’ allele is also added to the list of available alleles. The sum of the probabilities of all alleles is thus greater than one. In RMNE (and LR) calculations, this leads to a more conservative result. For each locus, the ‘observed’ alleles of each contributor are chosen from the list of available alleles based on the allele probabilities. Alleles that are more frequent in the population have a correspondingly higher chance to be chosen. Homozygous calls and contributors that have overlapping alleles are possible. Profiles were calculated for a 7 loci set (D3S1358, TH01, D21S11, D18S51, vWA, D8S1179, FGA) and a 14 loci set (D3S1358, TH01, D21S11, D18S51, vWA, D8S1179, FGA, SE33, D16S539, D10S1248, D1S1656, D12S391, D2S441, D22S1045). Supplementary file 3 contains the used allele frequencies.

## 3. Results and discussion

### 3.1. Computational requirements and performance

Fig. 1a shows the number of possible DO combinations for exactly 0 to 28 assumed drop-outs in 14 loci. For 1 DO, there are 14 possible combinations. For 28 DO there is only one combination possible. The highest number of combinations is possible when the number of assumed DO is the same as the number of loci. The corresponding single-core calculation times for the 2 developed Python scripts is also plotted in Fig. 1a. The calculation times and memory requirements of the faster, memory intensive script follows the number of possible combinations and thus reaches a maximum when the number of assumed DO is the same as the number of loci. The calculation time of the time intensive script



**Fig. 1.** (a) Number of possible DO combinations for exactly 0–28 assumed DO in 14 loci. The corresponding single-core calculation times for the 2 developed Python scripts is also plotted. (b) Analogous (cumulative) plot when allowing 0–28 DO.

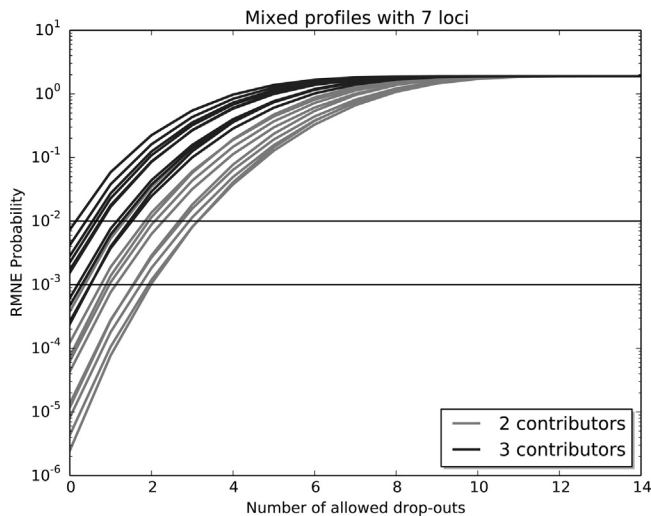
follows the formula for all combinations with repetitions and thus increases dramatically with the number of assumed DO. Fig. 1b shows the cumulative version of Fig. 1a, allowing for a number of DO instead of assuming an exact number of DO.

For analyses with a higher number of loci, it becomes unfeasible to routinely calculate RMNE values allowing for a high number of drop-outs. With the fast, memory intensive algorithm, the required memory becomes the limitation. With the time intensive algorithm, the calculation time becomes prohibitive.

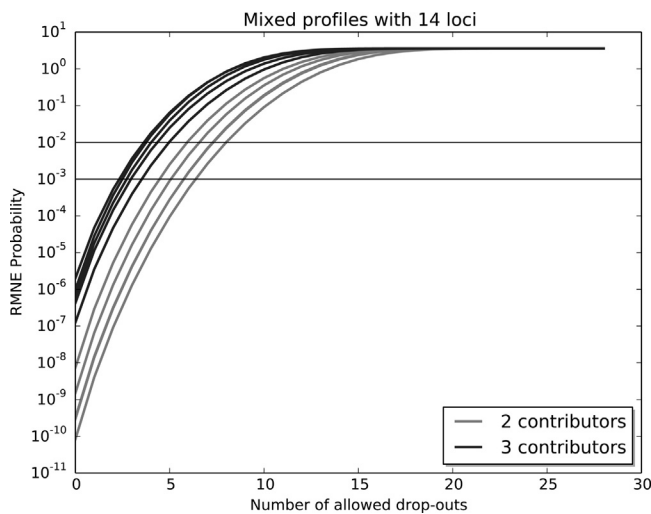
### 3.2. RMNE probability increases with increased number of allowed drop-outs

Figs. 2 and 3 show the RMNE probabilities of 7-loci and 14-loci profiles. The RMNE probability was calculated for random mixed profiles with 2 and 3 contributors, allowing up to the maximum number of allelic DO. The results show how the RMNE probability nears 1 when the maximum number of allowed drop-outs is reached. When 2 drop-outs are allowed for each locus, in theory the complete population is included because an evidence profile can maximally lack 2 alleles for each locus when compared to a suspect profile. The calculated RMNE value is a little higher than 1, because the sum per locus of the probabilities of all alleles is greater than one due to the use of a minimum allele frequency (see Section 2).





**Fig. 2.** RMNE probabilities of random 7-loci profiles with 2 (gray) and 3 (black) contributors. The horizontal lines show an arbitrary RMNE cut-off of 1 in 100 and 1 in 1000.



**Fig. 3.** RMNE probabilities of random 14-loci profiles with 2 (gray) and 3 (black) contributors. The horizontal lines show an arbitrary RMNE cut-off of 1 in 100 and 1 in 1000.

The non-logarithmic plots of the RMNE probabilities allowing a number of DO in a range from 0 to the possible maximum number of DO, resemble a sigmoid curve (Supplementary file 4). With each DO, the RMNE probability increases. The increments get bigger and bigger with additional allowed DO to become smaller again after a certain number of allowed DO is reached.

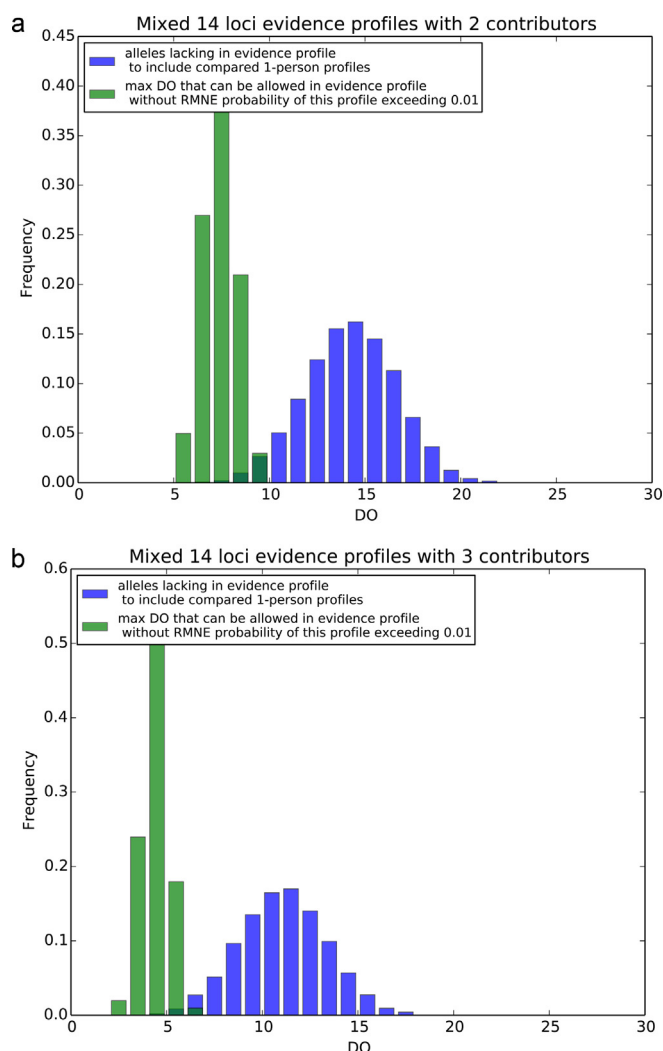
### 3.3. How many drop-outs can be allowed in RMNE?

An arbitrary RMNE threshold of 1 in 100 is often used as a threshold below which a profile is considered to have insufficient evidential value. For 7-loci profiles with at least 2 contributors, this threshold is reached for all generated profiles when 4 or more DO are allowed (see Fig. 2). For the 14-loci profiles, the same happens when 8 DO are allowed (see Fig. 3). Allowing that many DO, could include several relatives of a contributor to the evidence profile. The RMNE probability, hence the name, is the probability that a random man is also not excluded by the evidence when the evidence is interpreted in the same way. It cannot be used as a

measure for the probability that a relative of the suspect is included/excluded. This of course is also the case when allowing for DO, but extra care should be taken that the RMNE value is not misinterpreted in this regard.

The question remains how many DO can be allowed in an evidence profile. In the introduction 2 ways of managing DO are discussed in detail. A first way is to make this assessment based on the quality of the evidence profile. In a second way, the number of DO is determined as the number of alleles that lack in the evidence profile compared to the suspect, after an initial assessment if DO is plausible. In this new paradigm, the RMNE is calculated as if the suspect is included, with the number of allowed “drop-outs” that is needed to make this inclusion. The RMNE could be stated as “if we include the suspect in the evidence profile, 1 in  $x$  number of random men would also be included in this evidence profile”. The RMNE probability gives an understandable measure for the statistical significance of the match. An RMNE probability of e.g. one in hundred means that the chance that a random person would also match, is one in hundred (under the approximation that profiles from unrelated people are independent and that the allele frequencies perfectly describe the underlying population), independent from the number of allowed DO that was needed to be able to make this match. A RMNE threshold to filter meaningful, useful, non-random inclusions from inclusions with a too high RMNE value could be adjusted based on the setting: when comparing a couple of suspects to the evidence profile, it could be kept at 1 in 100 or 1 in 1000. When the evidence profile is compared against a databank containing e.g.,  $1.00E+06$  profiles, the cut-off should be lowered.

A databank comparison simulation was performed by generating 100 random 2-contributor and 100 random 3-contributor mixture (evidence) profiles and comparing them to 100 random single contributor profiles. Each profile contained 14 loci. Overall  $2 \times 10,000$  comparisons were made. Fig. 4 shows the histogram of the number of alleles that the evidence profiles were lacking to include the compared single person profiles. The figure also shows a histogram of the maximum number of DO that can be allowed in the evidence profiles without the RMNE probability exceeding 0.01. This maximum number of allowed DO averaged at 6.8 and 3.9 for the 2- and 3-contributor profiles, respectively. The average corresponding RMNE probability for that maximum number of allowed DO was 0.0053 and 0.0046, respectively. The number of random matches for 10,000 comparisons with that maximum number of allowed DO is thus 53 persons matching a 2-contributor profile and 46 persons matching a 3-contributor profile. For every comparison between an evidence profile and a single person profile, the RMNE value was calculated with the number of allowed DO needed to include the single person profile, accounting for all lacking alleles in the evidence profile. All single person profiles are thus included in all evidence profiles. When this RMNE value of the evidence profile was not exceeding 0.01, the compared person was incriminated. In the simulation, 8 persons were included in at least one 2-contributor profile and 11 were included in at least one 3-contributor profile. The discrepancy between the estimated number of random men not excluded and the actual figures can be explained by the conservative nature of the RMNE calculation. The calculation is conservative because the use of a minimal allele frequency (see Section 2) leads to an overestimation of the RMNE probability and thus an underestimation of the evidential value of a profile in terms of including a possible contributor. In above mentioned databank comparison simulation, the RMNE threshold of 0.01 is used for educational purposes. When performing 10,000 comparisons, a more meaningful RMNE threshold would be 0.0001. For this threshold none of the 100 random single person profiles is included in any of the 100 2- and 3-contributor profiles.



**Fig. 4.** Databank comparison simulation with 10,000 profile comparisons allowing for DO.

### 3.4. Conservative nature of RMNE allowing for drop-outs

Our RMNE approach [2], uses very little information: allele “present”/“not present”, number of allowed drop-outs and the allele frequency in the calculation. The calculation assumes that there is no linkage disequilibrium between the loci. No further assumptions are made, resulting in a conservative calculation. When there is uncertainty around the weight of evidence, the tendency is typically to be somewhat more conservative (i.e., to favor the defense) than liberal (i.e., to favor the prosecution). This is justified as the consequences of being too liberal could be disastrously unfair to a suspect. Our proposed method could be considered unduly conservative. Milot et al. [14] discusses the conservative nature of our method and propose a less conservative adaptation of the RMNE method that requires an assumption on the number of persons that contributed to the profile.

The conservative nature of our approach is due to several reasons, which are related to the fact that almost no assumptions are incorporated in the calculation: 1. It is not determined at which loci the drop-outs have occurred. 2. The RMNE value is not calculated for an exact number of drop-outs, but allows up to a number of drop-outs. By summing RMNE for  $r=0$ ,  $r=1$ ,  $r=2$ , etc., all these mutually exclusive possibilities are summed. If the number

of drop-outs could be exactly determined, this step could be avoided. 3. Allele frequencies have a minimal value. This avoids that too much evidential value is inferred from the presence of a rare allele and is a common practice in evidential value calculations (RMNE and LR).

One could debate if our method is not too conservative and the calculated RMNE thus deviates too much from the actual underlying evidential value. Our results section shows that using our approach on contemporary profiles (with 14 loci), even allowing for several drop-outs, often results in conservatively calculated RMNE values that are still smaller than one in millions. With the ever increasing number of analyzed loci such as the 200+ loci datasets using Illumina technology, RMNE probabilities allowing for drop-outs are infinitesimal (e.g., 5 DO in 3 contributor profiles with 210 loci results in RMNE values in the order of magnitude of 1 in  $10^E-200$ ; data not shown). While this does not show that our RMNE calculation closely estimates the true evidential value, it shows that our RMNE calculation provides a conservative estimation that can be used in a judicial process in the majority of the cases. The rapid decrease in evidential value when allowing for many drop-outs is partly due to the conservative nature of our calculation. In our opinion, our conservative approach forms a safeguard against wrong assumptions: wrong estimation of the number of drop-outs and the number of contributors, wrong estimation of the loci in which the drop-outs have occurred (sometimes biased through the knowledge of the suspect's profile), and wrong estimation of rare allele frequencies in the population.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fsigen.2015.08.001>.

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