2 CaseSolver: An investigative open source expert system based on 3 EuroForMix

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11 Abstract

12 For very serious crimes, reporting scientists often have to contend with complex cases where literally
13 hundreds of items are submitted by investigators for analysis. In order to efficiently expedite the
14 challenge of comparing reference profiles to evidence profiles, many of which are mixtures, we have
15 developed an investigative open source expert system *CaseSolver*. We have analysed a real case
16 based on *GlobalFiler* involving 119 evidence profiles and 3 reference profiles. To provide a
17 demonstration of the power of the system we also added the three references to a fictive large
18 database of 1 million individuals in order to test subsequent recovery of the presumed true
19 contributors. *CaseSolver* was used on a *Fusion 6C* validation study involving 25 two- to four-person
20 mixture profiles based on 14 reference profiles. The sequential use of simple allele comparison, the
21 qualitative model (*forensim*) and the quantitative model (*EuroForMix*) makes the analysis very fast
22 and accurate – and finally, the software generates a list of potential match candidates which can be
23 exported as a report. From these two studies we found that the resolution of match candidates from
24 *CaseSolver* was the same as that reported by a scientist who worked manually through the samples,
25 except that *CaseSolver* highlighted two manual errors. For the validation study we found low
26 template DNA samples giving negative results, which demonstrate the limitations of the tool; but
27 overall our assessment shows that *CaseSolver* will benefit all analyses involving mixture
28 interpretation and screening. Importantly, *CaseSolver* removes the very time-consuming aspect of
29 manual comparison and gives improved quality by preventing manual errors.

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31 Keywords: **DNA comparison, Likelihood ratio, Database searching, Expert system**

33 1. Introduction

34 Commercially available short tandem repeats (STR) amplification kits for forensic casework are
35 becoming more and more comprehensive as the numbers of STR loci are increasing. The first STR
36 multiplexes developed by the Forensic Science Service (FSS) in the United Kingdom in the 1990s
37 consisted of four (Quadruplex) and six (SGM) polymorphic STRs, which provided a matching
38 probability of approximately 1 in 10 000 and 1 in 50 million respectively [1, 2]. Since then,
39 commercial companies, such as Promega Corporation, Qiagen and ThermoFisher Scientific (former
40 Applied Biosystems and Life Technologies), have developed STR multiplexes for forensic purposes
41 with additional numbers of STR loci, increasing discrimination powers to about 1 in 1 quintillion
42 (1e18) for 15 STRs and 1 in 10 octillion (1e26) for 21 STRs [3]. However, given that such extreme
43 extrapolations of probabilistic models can never be validated, we limit our evaluative statements to 1
44 in 1 billion. In addition, improvements in biochemistry have resulted in the detection of very low
45 levels of DNA, typically described as low-template analysis [4, 5]. These tests can be described as
46 both universal and routine. The GlobalFiler™ PCR Amplification Kit, manufactured by ThermoFisher
47 Scientific and the PowerPlex® Fusion 6C system by Promega Corporation, consisting of 24 and 27
48 markers, respectively, includes both the traditional US and European STR loci [6, 7]. These

amplification kits provide even higher discrimination power, but also enable transatlantic exchange of DNA profiles. The increasing number of additional loci provides more data to manage and causes the interpretation and comparison of DNA profiles to become a time consuming bottle-neck. This can be a challenge in routine casework, especially if multiple stains produce different DNA mixtures of different complexity. Also, when there is little information available about the case circumstances, the list of potential suspects may be vast, as it could comprise individuals held on a national DNA database. The police will expect the scientist to provide investigative leads to identify potential suspects.

A number of statistical software packages are available for the purpose of evaluating the strength of the evidence by calculating the Likelihood Ratio (LR) [8-13]. But before this step is carried out, numerous cross-comparisons of complex crime-stain profiles with reference samples may be needed. The software named *eDNA* was developed to assist with this task [14]; it automatically provides an overview over all data in casework in a structured manner based on categorization and simple allele comparison. However, the software is not suitable for analysing very large cases or doing database searching, and it does not incorporate any possibility to carry out statistical analysis for mixtures. *SmartRank* is an open source software for searching DNA databases with complex STR profiles (available at <http://lrmixstudio.org>). The software implements the *forensim* model [15-17] that was adapted to enable fast and efficient searching of voluminous databases [18, 19]. *SmartRank* presents the top ranking candidate(s) that exceed a predefined LR threshold. Although the software has proven to be useful and efficient in forensic casework, it does not take peak heights into account and it does not provide a structured overview of evidence and reference profiles. Consequently, there is still a need for advanced data management tools that can provide an automated overview of all the evidence profiles (both mixtures and non-mixtures), where the peak height information is taken into consideration, in order to discover potential matches when a large number of references are provided.

In this paper we present *CaseSolver* (CS), a novel expert system, used to manage comprehensive casework data (open-source and freely available at www.euroformix.com/casesolver). CS provides a graphical user interface in the statistical software *R* [20] where it can perform rapid comparisons of reference DNA profiles from single source profiles and mixtures. CS follows the method presented by Bleka et al. [21] where a step-wise sequential analysis is performed: First a simple allele comparison is undertaken, followed by analysis with a qualitative model (*forensim* [15]) and finally a quantitative model (*EuroForMix* (EFM) [8], which is a similar model as *DNAmixtures* [13, 22]) is used in order to provide the ultimate list of candidate matches.

To demonstrate how the software can be used as an efficient tool to resolve candidate matches for reporting scientists, we applied CS on a real case (based on *GlobalFiler*) containing over 100 evidence profiles. In order to test CS more extensively, we generated a large database containing 1 million references and analysed these together with the real case data. Finally, we applied CS on a dataset based on a validation study of the *Fusion 6C* system conducted in Norway.

2 Data and experimental design

2.1 Circumstances and DNA profiles of a real case

Following a report from a concerned relative whose sister (REF1) had been missing for several days, the police forcibly entered her residence to find her deceased along with her son (REF2). There had clearly been a violent struggle where the victims had been stabbed multiple times. As the investigators had no indication of a suspect(s) it was decided to carry out comprehensive sampling of the crime scene in order to discover potential suspects from DNA analysis (a map of the crime scene

is presented in Fig S1 in supplement A.1). *Posteriorly*, a reference sample from a suspect (REF3) apprehended by the police was collected. A total of 119 crime stains were collected from 46 different items. The majority were blood stains, but contact traces were also recovered from cigarette butts, swabs of mobile telephones and handles of drawers and knife handles and blades that may have been used as weapons by one or more perpetrators and victims (see the list with items and the corresponding DNA profile names in Table S1 in supplement A.1). The DNA profiles were generated using *GlobalFiler* (ThermoFisher Scientific) and the analytical threshold (AT) was set to 70 rfu. More details about the generated profiles are given in supplement A.1. All the DNA profiles (alleles and peak heights) are given at <http://www.euroformix.com/datasets>. Also provided is a file of allele frequencies used to calculate the LR [23], based on 284 individuals analysed with *GlobalFiler*.

2.2 Searching a large fictive national DNA database

Often the candidate list of suspects may extend to national DNA databases. A portion of a database may be selected based on geographical location of the offence and known offenders within a given area. In order to test the efficiency of CS, the three profiles of the known persons of interest in the real case, i.e. the two victims and the suspect, were added to 1 mill. randomly generated reference profiles and these were compared to the evidence profiles (based on the crime-stains in the case) using CS. Here the *GlobalFiler* allele frequencies were used, but in addition we inserted a minimum frequency of 0.0018 for allele 5.3 and allele 28 at the SE33 locus (and afterwards all frequencies were normalized). Importantly, the generation of such a database enables us to count (and estimate) the number of false positives obtained.

2.3 A validation study based on *Fusion 6C*

The purpose of the validation study was to investigate how DNA profiles based on the PowerPlex® *Fusion 6C* (Promega) kit performed with regards to the number of known contributors, mixture proportions, allele drop out, artefacts (stutters/drop in) and mixture interpretation, both manually and with LR calculations using *EFM* (quantitative model) and *LRmix Studio* (qualitative model). We applied the samples from the validation study as a case in CS to determine its performance, comprising nine 2-person mixtures, twelve 3-person mixtures and four 4-persons mixtures. These were constructed from 14 good quality single source crime stain samples in different mixture proportions. The 14 single source stains acted as reference samples. The analytical threshold (AT) was set to 100 rfu. More details about the profiles are given in supplement A.2. The allele frequencies used to calculate the LR values were based on the Norwegian database found at <http://www.familias.no>.

3 Method

3.1 Initial analysis

When CS imports the data (both alleles and peak heights), the software first estimates the minimum number of contributors using the simple rule $K = \text{roundUp}(\text{'maximum allele count for any markers'}/2)$ [24], where *roundUp* means rounding up to closest integer. Based on this rule, it is possible to assign whether an evidence profile is most likely to be a single source (SS) profile ($K=1$) or a mixture ($K>1$). A consequence is that artefacts creating additional alleles like stutters or drop-ins may overestimate the number of contributors. After carrying out 'the number of contributors' classification, CS compares all the imported references against all evidence profiles which were classified as SS profiles (i.e. evidence profiles with $K=1$). If the alleles of the reference (non-empty loci) match with all the alleles of the SS profile, then CS tags the reference name under the "MatchStatus" column of the evidence profile. In order to take into account partial profiles, the user can set a threshold to determine the number of non-empty loci in the SS profile to designate "a

match” (seven is set as a default). If no reference matched the SS profile, CS creates an “unknown” reference profile with a specific ID. If several of these unknown SS profiles are identical, these will have the same unknown ID. The “MatchStatus” for mixtures is always given as “mixture” (Fig 1).

The screenshot shows the 'Data' panel of the CS software. At the top, there are tabs for 'Data', 'Match matrix', 'Match list (Qual LR)', 'Match list (Quan LR)', 'Mixtures', and 'Deconvoluted'. Below the tabs is a 'Select Case ID' section with a 'Change view' button, a dropdown menu set to 'RealCase1', an 'Import' button, and two dropdown menus for 'Sort Evids by #' and 'Sort Refs by #'. The main area is titled 'Evidence profile(s) and Reference profile(s)'. It contains two tables. The top table lists evidence profiles (ID #1 to #10) with columns for SampleName, MatchStatus, and various genetic markers (D3S1358, VWA, D16S539, CSF1PO, TPOX, YINDEL, AMEL, D8S1179). The bottom table lists reference profiles (ID #1 to #3) with columns for SampleName and the same genetic markers. At the bottom, there is a 'Functionalities' section with buttons for 'Compare', 'Create Report', 'Add/Edit references', 'Export profile(s)', 'Calculate RMP', 'Calculate IBS', 'Deconvolve', and 'RESTART'.

ID	SampleName	MatchStatus	D3S1358	VWA	D16S539	CSF1PO	TPOX	YINDEL	AMEL	D8S1179
#1	19.01	mixture	15						X/Y	12/13/14
#2	40.03	mixture						2		13/14
#3	08.01	mixture	14/17	15/16				2	X/Y	11/12/13
#4	13.02	mixture	14/15/17	15/16	11/12	9/10		2	X	12/13/14
#5	20.01	mixture	14/15/17	14/18				2	X/Y	12/13/14
#6	30.01	mixture	15/17	15/16	11/12	9/10	7		X	12/13/14
#7	37.01	mixture	15/17	15/16	11/12	9/10	7		X	12/14
#8	38.15	mixture	15/17	15/16	11/12	9/10	7		X	12/14
#9	02.01	REF3	14/15	14/18	8/12	9/11	7	2	X/Y	13/14
#10	07.01	REF3	14/15	14/18	8/12	9/11	7	2	X/Y	13/14

ID	SampleName	D3S1358	VWA	D16S539	CSF1PO	TPOX	YINDEL	AMEL	D8S1179
#1	REF1	15/17	15/16	11/12	9/10	7/7		X/X	12/14
#2	REF2	14/17	15/16	11/12	10/11	7/7	2	X/Y	12/12
#3	REF3	14/15	14/18	8/12	9/11	7/7	2	X/Y	13/14

Fig 1: The figure shows the “Data” panel of CS after importing the real case example (“RealCase1”). Here the tables visualize the alleles for each of the profiles, with evidence profiles given in the upper table, and the reference profiles given in the lower table.

3.2 Mixture comparison

The total number of comparisons in a case is ‘the number of references’ multiplied by ‘the number of mixtures’, and the time taken to perform such a number of comparisons relies heavily upon on the method used. Simple allele comparison, also called “Matching Allele Count” (MAC) [25], is extremely rapid to perform (seconds), whereas inference of the qualitative model would be slower (minutes), and the inference of the quantitative model slower still (hours) [21]. Hence, in order to save computing time, the mixture comparison in CS is based on a three step approach, as described by Bleka et al. [21]. Each step produces a selected list of candidates from the previous step, which are based on user specified thresholds. The first step performs MAC, followed by LR calculations based on the qualitative model (*forensim* [15]). Finally, LR calculations based on the quantitative model (*EFM* [8]) were used to filter the list of candidates. Mathematical details for the different models are provided in supplement B.1. We recap how the LR is calculated for both the qualitative and quantitative model:

Consider the two investigative propositions, assuming K number of contributors:

H_p : “Person of interest (POI) and $K - 1$ unknowns (unrelated) are contributors to evidence profile E ”

H_d : “ K unknowns (unrelated) are contributors to evidence profile E ”

The LR is given by the expression $LR = \frac{P(E|H_p)}{P(E|H_d)}$. To calculate this, we apply a statistical model $P(E|\beta, H)$, with unknown parameters β (with H either H_p or H_d). By using the maximum likelihood estimate (MLE) approach to infer the unknown parameters [8, 13], the LR is given as:

$$LR = \frac{\max_{\beta} P(E|\beta, H_p)}{\max_{\beta} P(E|\beta, H_d)} = \frac{L_p}{L_d}$$

For the qualitative model, β is the parameter giving the probability of an allele dropout (considered as the “basic model”) [10, 17]. Here the evidence profile data (E) are the presence/absence of the alleles. For the quantitative model (EFM), the peak heights (P.H.) of each allele are also considered as part of E , and here β is the set of following parameters: “Mixture proportions”, “P.H. expectation”, “P.H. variability”, “Degradation slope” (if turned on), “Stutter prop.” (if turned on). The MLE approach means that these parameters are selected such that they maximize the probability of observing the evidence profile E . The number of contributors, K , is estimated using the method based on the qualitative model as presented in the Appendix of Bleka et al. [26]. Here the number of contributors is optimized using ‘ $\ln L_d - K$ ’ as a model selection criterion, initially with $K = 1$.

For both the qualitative and the quantitative models, allele frequency data were used to inform the genotype probabilities. Unobserved alleles were assigned with the minimum observed allele frequency (0.0018 for *GlobalFiler* and 5.2e-05 for *Fusion 6C* frequencies).

3.2.1 Simple allele comparison

The first step to compare references against mixture profiles is carried out by applying the MAC. In CS, MAC is given as a percentage of the alleles of the reference that are included in the evidence profile (given as a decimal format). Empty loci are ignored. This method does not use the allele frequencies, information of the peak heights or the detection threshold indicated in the model. The results for all comparisons are present in the panel *Match matrix* (see Fig S4a in supplement C.1). A user specified “MAC threshold” is utilised to select the candidates for the next comparison step which is based on LR. The default threshold in CS is 0.8 (this corresponds to a 20% mismatch with the evidence profile), making it potentially possible to detect contributors with a lot of dropouts in low-template evidence profiles. Depending on the application, it is for the user to decide the value of the threshold.

3.2.2 Comparisons based on LR

In the second step (which is based on applying the qualitative model), the LR is calculated for each candidate generated from the first step: Here, the number of contributors (K) is estimated for each evidence profile, and then the corresponding candidate reference profiles are specified in turn as the POI (under definition of the H_p proposition). The LR results are then listed in the panel *Match list (Qual.LR)*. Here, the “Qual. LR threshold” is specified by the user in order to select the candidates for the next comparison step (step 3) which is based on the quantitative model. In the third step, the LR for each candidate passing the threshold defined from the second step is re-calculated based on the quantitative model; the number of contributors is defined as described for the second step. The LR results are then listed in the panel *Match list (Quan.LR)*; the user specified “Quan. LR threshold” is utilised to indicate the final list of candidates which is provided in the panel *Mixtures*. See Figs S4 and S5 for the different panels in supplement C.1.

It is also possible for the user to either skip the step which is based on the qualitative model (step 2), or the step which is based on the quantitative model (step 3). Also, the user can, at any time after running step 2, calculate the quantitative based LR for a specific comparison with a user specified proposition set; he/she can condition on a reference (under both H_p and H_d) which is given as a match candidate in the *Mixtures* panel, and specify the number of contributors K . This is very useful in order to improve the estimate of the LR when moving from investigative to evaluative reasoning.

3.2.3 Pre-specified model parameters/configurations

The models used to calculate the LR requires the user to specify some parameters which are not optimized by the software. For both the qualitative and quantitative models he/she must specify the drop-in probability. For all analyses we used *drop-in probability* equal to 0.05. For the quantitative model we used the drop-in peak height parameter λ equal to 0.01, and the *detection threshold* (AT)

and *kit* as specified for the particular dataset. CS also allows the possibility to set several model configurations: Whether to apply degradation or/and stutter model. In our analysis we always used the degradation model but not the stutter model, since this increases computation time, and instead, a stutter filter was applied (see Appendix of Bleka et al. [26] for performance comparison).

The “*advanced options*” makes it possible to specify the maximum number of contributors (maxK) that can be investigated for either the qualitative/quantitative models. Either three or four contributors were used as defaults and the results were compared to determine the effects on the LR. Also, the number of optimizations used for the quantitative model is configurable (we recommend 4 optimizations).

As opposed to the interpretation software *LRmix* and *EFM*, the user of the current CS version (1.4.0) cannot specify the θ -correction in order to take sub-population structure into account [27] – this is fixed to zero in order to reduce computation time. However, the θ value is typically low and has little effect on the LR. Once candidate matches have been found, evaluation of the evidence will proceed according to laboratory protocols and a weight of evidence will be provided with the desired θ value (for instance by using *EFM*).

3.3 Deconvolution and other functionalities

In addition to calculating LR, CS is also capable of performing deconvolution based on the quantitative model (see supplement B.2 for mathematical details). Here the marginal probability of an unknown genotype is estimated for each marker and each contributor. CS lets the user define the “*Prob-ratio to next*” threshold (we used 10 as threshold); such that a genotype for an unknown contributor is deduced when the ratio between the top genotype and the second-top genotype is above this threshold. As a result, suggestive “unknown” profiles are present in the panel *Deconvolution* (see Fig S3 in supplement C.1). Here, the information about estimated mixture proportion and conditional references, are also present. The user can later extract this profile to the “reference profile” list for further comparisons against other mixture profiles. See supplement B.3 for more details about deconvolution and for an overview of other functionalities in CS.

3.4 Performance of database search

To quantify the performance of CS (as a test) we need to summarize false positives, i.e. non-contributors obtaining results above a specified threshold, and false negatives, i.e. contributors with results below a specified threshold.

When searching the large DNA database, we calculated the expected number of false positives to be obtained for our defined allele matching threshold (based on the allele frequencies) [28]. This was calculated for each evidence profiles by using the function *exactMACdistr* in the R-package *euroformix*. In addition, we quantified the time taken and the internal memory required to perform different steps in CS (i.e., importing, visualization and comparison of the data). We did this for various database sizes in order to find the technical limits of CS.

3.5 Comparison with manual investigation

In order to verify the benefits of CS we compared the time taken for the software to carry out the full analysis with the approximated time taken to carry out the manual investigation by the forensic scientists. The manual investigation was carried out by first visual inspection of the electropherograms and then all alleles in each marker in the evidence samples were compared to the alleles in references samples.

3.5 Hardware and R-packages

All analyses were run on a laptop with Intel Core i7-6600U CPU with 32 GB internal memory and a 500 GB internal SSD. To run CS we used the casesolver_1.4.0 package in the R x64 3.5.1 software. The graphical user interface of CS requires the packages gWidgets2_1.0-7 and gWidgets2tcltk_1.0-6, whereas the packages euroformix_2.1.0 and forensim_4.3 are required for calculation. The package igraph_1.2.2 is used to create the node graph and the package R2HTML_2.3.2 is used to create the final reports (in HTML format).

4 Results

4.1 Analysis of the real crime case

4.1.1 Import

In order to consider samples with sufficient quality we required at least 7 non-empty markers (all types of markers). Sample **16.03** was negative with all markers empty whereas samples **06.01**, **10.01**, **16.05**, **16.13**, **18.01**, **26.01** contained less than 7 markers. These were removed from the analysis. Some samples contained data from very few markers: The samples **19.01**, **40.03** and **08.01** (Fig 1) had 8, 10 and 14 non-empty autosomal markers, respectively.

4.1.2 Comparison against single source profiles

68 of the total 112 evidence samples imported were classified as SS profiles ($K = 1$). In this case, two (related) reference profiles which were the victims, REF1 and REF2, had been sampled (sharing 27 alleles) and many of the SS profiles matched these (29 samples matched REF1 and 17 samples matched REF2). CS was also able to assign 22 of the SS profiles as an unknown male profile (*Unknown 1*), which subsequently matched a known individual who had previously inhabited the flat (REF3). The remaining 44 evidence samples were categorized as mixtures (K was at least 2).

4.1.3 Comparison against mixtures

As a starting point for the analysis, MAC=0.8 and LR=1000 were used as LR thresholds (for both model types). From this analysis profiles **40.03**, **19.01** and **01.01** did not give any candidate matches. We were able to extract a full major profile, MAJ, from sample **01.01** using the deconvolution function (assuming $K = 2$). This profile was again compared to all of the other mixtures using the same thresholds. The first step provided a list of 76 comparisons with a MAC above 0.8 (176 comparisons completed within 1 s), whereas 73 of these had a qualitative LR above 1000 (completed in 21 s). Out of these, 70 comparisons had a quantitative LR above 1000 (completed in 2.8 hrs, but restricting the maximum number of contributors to three reduced it to only 10 min). Table 1 shows the resulting list after running through all these analyses with the corresponding match tree graph presented in Fig 2.

Sample Name	#contr (by view)	References	MixProp (%)	MAC (%)	Qual. LR (log10)	Quan. LR (log10)
01.01	2 (1?)	MAJ	91	100	16.5	29.4
03.01	2	REF2/REF1	72/28	100/100	18.5/18.8	29.4/18.7
03.02	2	REF2/REF1	79/21	100/98	17.3/17.2	29.5/13.0
04.01 (maxK=3,4)	3 (4) 4 (4)	REF1/REF2/REF3/MAJ REF1/REF2/REF3/MAJ	- 47/23/21/8.7	100/100/100/98 100/100/100/98	10.3/9.9/7.6/8.3 10.3/9.9/7.6/8.3	28.3/16.2/13.5/11.6 28.1/16.2/13.4/10.8
05.01	2 (1)	REF3	87	100	16.1	20.7

08.01	2	REF2	79	100	8.6	13.6
13.01	2	REF1/REF3	85/15	100/98	17.1/11.9	30.9/19.9
13.02	2 (1)	REF1	89	100	14.6	21.6
14.01	2 (1)	REF3	98	100	16.2	26.2
16.01	2 (1)	REF3	95	100	15.8	26.2
16.02	2	REF3/REF1	88/12	100/83	14.7/6.0	26.1/11.7
16.03.R	2 (1)	REF3	98	100	16.2	26.2
16.09	2	REF3	84	100	15.6	25.9
16.11	2	REF1/REF3	43/57	100/100	17.0/12.6	18.2/15.3
16.11.R	3	REF3/REF1/ REF2	45/37/17	100/100/100	10.2/13.3/12.6	13.5/18.7/12.7
16.12	2 (1)	REF1	88	100	18.7	27.6
17.01	2	REF3	83	100	15.4	22.4
20.01	2 (1)	REF3	93	0.97	11.8	20.0
21.02	2 (1)	REF3	97	100	16.1	26.2
21.03	3	REF3/REF1/REF2	62/25/13	100/100/98	10.3/13.3/10.0	18.3/17.6/10.9
26.01.R	2 (3?)	REF1/REF2	80/20	100/100	18.5/18.2	30.9/10.8
30.01	1	REF1	100	100	27.3	30.8
30.01.R	3 (2?)	REF1/REF3/REF2	65/31/4.4	100/100/90	13.2/10.1/6.7	26.5/16.9/6.5
37.01	1	REF1	100	100	27.0	30.9
37.03	2	REF1/REF2	51/49	100/100	18.7/18.3	25.3/23.7
38.06	2 (1)	REF1/REF2	89/11	100/86	18.4/9.7	30.9/4.7
38.15	2 (1)	REF1	97	100	19.2	30.9
39.01	2	REF2/REF1	76/24	100/95	18.3/16.6	28.8/16.2
39.04	2	REF2	91	100	17.9	29.6
39.05	2 (1)	REF2	91	100	17.6	29.6
39.06	1	REF2	100	100	26.4	29.6
39.07	2	REF1/REF2	72/28	100/100	18.8/18.5	30.5/18.8
39.08	2 (1)	REF2/REF1	91/8.8	100/86	18.4/12.5	29.6/4.7
39.09	2	REF1	96	100	18.2	30.9
40.01	2 (1)	REF2	92	100	17.6	29.6
40.02	2 (1)	REF2	97	100	18.9	29.5
40.04	2 (1)	REF2	95	100	18.9	29.5
40.05	3	REF3/REF1/REF2	62/22/16	100/100/98	10.4/13.5/11.5	19.8/17.8/12.2
40.05.R	3	REF3/REF2/REF1	45/33/22	100/100/100	10.4/12.9/13.4	12.9/16.0/16.0
41.01	2	REF2/REF1	88/12	100/90	16.0/10.8	29.6/4.0
41.01.R	3	REF2/REF3/REF1	70/14/16	100/100/100	12.5/10.2/13.2	29.5/15.2/10.8
45.01	2	REF1/REF2	56/44	100/100	18.7/18.3	26.9/22.2

Table 1: The table shows the match candidates from CS based on the three-step search strategy with MAC=0.8 and LR=1000 as thresholds. The column “#contr” indicates the number of contributors estimated by CS, with manual inspected given in parenthesis. The question mark indicate that the manual inspection is highly uncertain. The mixture proportion is estimated by conditioning on all matching references (at the same time) and “#contr” as the number of contributors. Candidates with

321 LR values between 1e3 and 1e6 are indicated in gray. Sample **04.01** was evaluated with both 3 and 4
 322 contributors (given by “maxK”).
 323

Matches for RealCase1

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 325 Fig 2: The figure shows the graph plot generated from the final match list (quantitative log10LR=6
 326 used as threshold), where the thickness of the edges and the distance between the nodes are
 327 inversely proportional to the quantitative based LR values (on log10 scale). Green nodes are
 328 reference profiles, cyan nodes are single source profiles, whereas the orange and red nodes are
 329 mixtures; orange nodes are mixtures with 2 contributors, and red nodes are mixtures with more than
 330 2 contributors (estimated).

331
 332

333 4.1.4 Further inspections

334 CS has the functionality of (suboptimal) testing for relatedness by comparing the number of shared
 335 alleles between references that are identical by state (IBS). For this, CS can calculate the distribution

of IBS under the assumption that references are unrelated (see table S3 in supplement C.2). This distribution is useful for testing whether two references are related or not. In the real case example we calculated IBS between 4 references (6 pairwise comparisons), and found that $IBS \geq 20$ was a threshold that gave a p -value less than 5% against of being unrelated ($6 \times 3.4e-3 = 0.02$). REF1 shared 27 alleles with REF2 (p -value= $7.3e-7$), whereas MAJ shared 24 alleles with REF3 (p -value= $1.2e-4$). When there is limited information in a case, information on potential relatedness would be useful to investigations.

In addition to the standard search strategy we considered a more thorough analysis to discover potential false negatives or positives (see supplement C.2 for more details about comparisons that were carried out). Here we lowered the MAC threshold to 0.7 and the qualitative based LR threshold to 1, and then applied the quantitative model where we conditioned on presumed matching profiles. Consequentially, we obtained the following (updated) results in about 1 hour of work using CS:

Potential false positives:

- **30.01.R – REF2** (MAC=0.9):
 - Conditioning on REF1 with K=3 contributors, reduced LR from $10^{6.4}$ to $10^{(-0.2)}$
 - Conditioning on REF1/REF3 with K=3 contributors, reduced LR further to $10^{(-1.7)}$
- **38.06 – REF2** (MAC=0.86):
 - Conditioning on REF1 with K=2 contributors, reduced LR from $10^{4.6}$ to $10^{(-4.1)}$
 - Conditioning on REF1 with K=3 contributors, reduced LR from $10^{4.6}$ to $10^{(1.7)}$

Potential false negatives:

- **39.04 – REF3** (MAC=0.76): Conditioning on REF2 with K=2 contributors, returned LR= $10^{(9.4)}$
- **41.01 – REF3** (MAC=0.79): Conditioning on REF2 with K=2 contributors, returned LR= $10^{(8.7)}$
- **40.03 – REF3** (MAC=0.75): Conditioning on none with K=2 contributors, returned LR= $10^{(4.0)}$

REF3 has allele 9 in marker DYS391, which was also obtained for all the three mixtures, increasing the support that REF3 could be a contributor (however this was not taken into account in the LR calculation).

4.1.4 Comparison with manual investigation

It was very difficult to calculate the time spent on the manual work (revision of data and comparison) since this was carried out as the samples are analyzed. A rough approximation on the time taken was 8 hours: The time taken for the comparison depended on the complexity of the evidence profiles. In general, when these are SS profiles with good quality, the comparison is easy. Comparison with higher order mixtures took much longer time and sometimes there were no clear conclusions. In addition, as some profiles come from related individuals (two of the references shared many alleles), the probability of making a mistake was high.

4.2 Managing and searching of a large fictive national DNA database

One million randomly generated reference profiles were imported into CS along with the real case data. We monitored the system requirement in terms of speed, memory and storage (see table S7 in supplement C.3 for detailed information of different database sizes). The time taken to simulate, import and visualize the data was about 14 min, with a high internal memory requirement of 13.4 GB. Storing the project required a file of 50 MB which could be imported in about 10 min. The time taken to compare all 1 mill. references (incl. case refs.) against the 44 classified mixtures with the MAC method (44 mill. comparisons in total), and generating the results in a huge table, was 34 min and required 12.5 GB. Of these, 56,521 comparisons satisfied the threshold of MAC=0.8. We next calculated the qualitative LR for all candidates (completed in almost 8 hours). A total of 302 of the comparisons satisfied a qualitative LR of at least 1000. Calculating the quantitative LR for these

remaining candidates took 16 min/4 hrs (maxK=3/4), and resulted in 210/190 match candidates with LR above 1000 (maxK=3/4) and 68 with LR above 1e6. Table 2 gives an overview of the number of generated references found after applying the “threshold test” in each of the comparison steps in CS. The presumed four-person mixture profile **04.01** yielded the most false positives due to the high number of observed alleles, whereas the qualitative LR method removed most of these with a threshold LR=1000.

In the next step, the quantitative model reduced the number of candidates further; however, many candidates still had a LR above 1000. For the presumed four-person mixture profile **04.01** the number of false positives was increased from 7 to 27 when changing from three to four contributors in the quantitative model; here the LR of the top ranked generated reference was increased. The largest false positive quantitative based LR for a randomly generated reference was log10LR=6.1 (MAC=0.94), obtained for the low quality sample **19.01**. This was the only comparison with a LR above 1 mill. Hence all the comparison results for the known references in the previous section (Table 1) obtained the highest rankings when a LR threshold of 1 mill. was used.

Sample Name	Expected	MAC=0.8	Qual. LR=1000	Max Qual. LR (log10) w/MAC	Quan. LR=1000	Quan. LR=1e6	Max Quan.LR (log10) w/MAC
04.01 (maxK=3/4)	44567	44397	41	5.3 (0.95)	7/27	0/0	4.8 (0.95)/5.9 (0.95)
16.11.R	2691	2665	12	4.6 (0.90)	2	0	4.8 (0.88)
41.01.R	2456	2466	10	5.9 (0.93)	6	0	5.0 (0.86)
30.01.R	1616	1639	24	4.8 (0.88)	23	0	4.5 (0.86)
21.03	1183	1200	21	5.3 (0.90)	13	0	4.7 (0.86)
19.01	1170	1190	35	4.5 (0.94)	32	1	6.1 (0.94)
40.05	1120	1182	17	5.3 (0.90)	8	0	4.6 (0.86)
40.05.R	1073	1100	17	5.3 (0.90)	6	0	3.9 (0.90)
16.11	226	251	0	-0.4 (0.81)	-	-	-
40.03	114	94	24	4.7 (0.85)	22	0	5.5 (0.85)
13.01	83	82	0	2.4 (0.81)	0	0	5.3 (0.83)
16.02	72	65	0	2.8 (0.81)	-	-	-
08.01	43	57	26	4.6 (0.82)	21	0	4.7 (0.82)
41.01	35	41	1	3.6 (0.81)	0	-	-4.2 (0.81)
39.04	11	13	1	3.4 (0.81)	0	-	-2.0 (0.81)
38.06/16.09/39.05	1.4/0.6/0.4	All 1	All 0	2.0 /1.0/2.5 (all 0.81)	-	-	-

Table 2: The table gives an overview of the observed number of generated references (out of 1 mill. total) found after applying the filter in each of the comparison steps in CS, for each evidence profile. “Expected” is the expected number of references which are expected to satisfy the MAC=0.8 threshold. “. Here, only evidences with at least one generated reference candidate, are presented. For sample 04.01 we evaluated both for K=3 and K=4 number of contributors for the quantitative model. The last column presents the maximum observed LR for the generated reference profile with the observed MAC presented in parentheses.

4.3 Analysis of the *Fusion 6C* validation study

In this section we show the results of using CS on data from the validation study (25 mixtures and 14 references) where the ground truth is known (either the reference is a contributor or a non-contributor). Here, a number of false positive and negative results were observed out of 350

comparisons – the number was dependent upon the values of the “MAC threshold”, “Qual.LR threshold” and “Quan.LR threshold”. The results are presented in Table 3. False positives were only observed using the MAC method (comparison **S00_B03 – R15** were 0.91 was the highest observed), whereas both the qualitative/quantitative based methods eliminated all instances. The highest qualitative based LR for a non-contributor was log10LR=-2.3 (comparison **S01_B01 – R23**).

Table 3 shows that different threshold settings influence the number of false negatives, but has little effect on the number of false positives. The best strategy can be defined as the one which minimizes numbers of false positives and negatives – using “MAC threshold”=0.7 and “Qual.LR threshold”=1 without using the quantitative based method (see Fig S2 in the supplement A.2 for a graph plot based on the final match list). Here only one false negative was observed: **S02_B01 – R7**, where the reference had MAC=0.74 (12 dropouts) and the qualitative log10LR=-2.2 ($K = 2$). The number of contributors for this evidence profile had been underestimated. For the same sample and for the same reason, the quantitative model additionally returned **S02_B01 – R15** as a false negative having MAC=0.85 (7 dropouts) and quantitative log10LR=-4.7 ($K = 2$). When the true number of contributors was conditioned on ($K = 3$), the quantitative based method gave log10LR=12.6 for R7 and log10LR=4.2 for R15.

The quantitative based method returned four false negatives with four-person mixtures, where the number of contributors were only considered as three: The comparisons **S00_B01 – R5/R10** and **S00_B04 – R5/R10**. CS estimated these mixtures as four contributors but there was, by default, an upper limit for the quantitative model of three contributors imposed, in order to speed-up calculations. Setting four contributors as a maximum (instead of three) solved this issue.

MAC	Qual.LR	Quan.LR (maxK)	Time	#Comp. qual/quant	False Positives	False Negatives
0.8	1000	-	2 min	82/-	17/0/-	5/8/-
0.8	1	-	2 min	82/-	17/0/-	5/5/-
0.8	1	1000 (3)	26 min	82/65	17/0/0	5/5/10
0.8	1	1000 (4)	12.5 hrs	82/65	17/0/0	5/5/6
0.8	1	1 (3)	26 min	82/65	17/0/0	5/5/9
0.8	1	1 (4)	12.5 hrs	82/65	17/0/0	5/5/6
0.7	1000	-	2 min	124/-	54/0/-	0/7/-
0.7	1	-	2 min	124/-	54/0/-	0/1/-
0.7	1	1000 (3)	26 min	124/69	54/0/0	0/1/6
0.7	1	1000 (4)	12.5 hrs	124/69	54/0/0	0/1/2
0.7	1	1 (3)	26 min	124/69	54/0/0	0/1/5
0.7	1	1 (4)	12.5 hrs	124/69	54/0/0	0/1/2

Table 3: The table shows the number of false positives/negatives for different sets of thresholds (for each row). MAC is the threshold of proportion of matching alleles of references whereas “Qual.LR” and “Quan.LR” are the LR thresholds for the qualitative and quantitative based methods, respectively. “maxK” is the maximum number of contributors allowed. “#Comp.” is the number of comparisons when performing either the qualitative based method or quantitative based method. The format x/y/z means that x, y and z is the number of false positives/negatives after sequentially applying the “MAC threshold” (x), the “Qual.LR threshold” (y) and the “Quan.LR threshold” (z). “-” means that the method was not applied.

5 Discussion

5.1 The need for digital tools in forensic genetics

The rapid change and use of DNA technology requires new analytical software to be developed in order to deal with vast amount of information generated. Traditional DNA analyses are often based

on manual comparisons of alleles, together with peak heights, using pen and paper. As the number of markers increase, such work requires a lot of resources and transcription errors are a particular concern. The increased sensitivity of DNA typing has also led to the generation of more mixture profiles, and analysing these together with a large number of other profiles in complex cases is required by routine caseworkers.

In this paper we have introduced an exploratory investigative tool to filter large sets of data in order to discover candidate matches (potential matches between reference profiles and evidence profiles), either single source profiles or mixtures. We provide a demonstration of a challenging case, and we also show the benefit of being able to perform more thorough analyses (LR calculation, deconvolution or simple relatedness testing) where hypotheses can be specified more carefully, without exporting to other software.

5.2 Improving the efficiency of complex investigations

With complex investigations there are often no suspect(s). Under these circumstances, investigators will usually resort to a search of the national DNA database in order to discover potential candidates. Whereas this is quite a straightforward process for single, well represented DNA profiles, when the evidence profiles are mixtures a different approach is needed. Most national DNA databases are geared to work with single source profiles. If a mixture is obtained then deconvolution may be carried out in order to extract a candidate reference to search with, but this procedure is problematic because it is realistically restricted to clear major contributors; if the person of interest is a minor contributor, the approach does not work because masking and drop-out means that there can be no definitive reference sample to search with. As a result, reliance upon deconvolution is inefficient as many crime-stains cannot be analysed. Consequently, many cases remain unsolved.

The likelihood ratio approach employed by probabilistic models is a preferable way forward because there is no restriction on profiles that can be searched (no deconvolution) so that minor contributors can be investigated – there is no need for a clear major contributor to be the person of interest. Such an approach is employed by *SmartRank* [18]. This is a rapid search engine that employs the qualitative model of Haned et al. [17]. However, quantitative models are more informative because they take account of the peak height information in the evidence profile. But the downside is that the computational requirements are much greater. Consequently, this may act as a limitation on the number of reference samples that may be searched. In order to solve this problem, we have devised a three-step test. Clearly, there is no point in carry out extensive computations on samples where only a few alleles match. Therefore, these can be quickly filtered by carrying out simple allele matching and eliminating those references that fall below a threshold. The remainder can be searched by the qualitative model, and the final list refined by the quantitative model.

The investigative procedure is outlined in Fig 3. A list of reference profiles to search may arise from staff elimination databases (contamination search); mass screens of local populations; national DNA databases; or persons of interest highlighted as a result of other investigations. The purpose of the lab is to compare the reference profiles against the relevant evidence profile(s) to provide a ranked list of candidates. This list is forwarded to the police who carry out further investigations. The list of candidates may be restricted according to criteria such as age or geographic location [29]. If phenotyping information is available e.g. geographic ancestry or hair/eye colour, then this can also be used to reduce the size of candidate lists [30], noting that no test is 100% definitive hence there is a risk of false negatives.

Either all candidates are eliminated, or one is identified as a suspect who is carried forward to the prosecuting authorities for evaluation based on additional evidence in the case. If all are eliminated,

the police contact the lab and request an extension to the candidate list, either including references with lower ranking, or by considering a new set of references based upon new criteria.

When there is sufficient non-DNA evidence in the case to proceed with the prosecution, then the scientist switches to evaluative mode as reviewed by Gill et al. [31]. Once in the evaluative mode the strength of evidence is considered using refined propositions that are posited by the relevant authorities [32], and with suitable ' θ -correction' (the current version of CS does not consider ' θ -correction') and other settings discussed in Bleka et al. [8]. Importantly, CS can export the selected DNA profiles directly to EFM, or to text files for other software users, so that there is no method restriction for the evaluative phase to take place.

The idea of investigative searches is to prioritise police resources where there is limited information in the case. If the DNA evidence profile is non-complex and well represented at 16+ loci then there is usually no issue with searching very large reference databases of several million individuals. The risks of missing someone are low. However, when the profile is a complex mixture of three or more persons then it is inevitable that there are many false positive matches in the candidate list. In order to carry out an investigation, this list is ranked according to the likelihood ratio, and the police will investigate the individuals in this ranked order. In order to forward a person to the evaluative stage, it is necessary to have additional evidence e.g. eye witness, fibres. It is therefore a time-consuming process. For example, if no candidate is found after the first one hundred¹ comparisons, then there is an option to extend the list to the next one hundred, and so-on. This process is dictated entirely by the police investigator and it is ultimately dependent upon the resources and time available to investigate a particular crime. There will be more resources for a serial killing than for a petty burglary. In addition, rather than search entire databases, subsets of databases may be prioritized based on, for example, geographical location, age and sex of proposed offender; previous convictions of offenders with a known modus operandi etc. These are examples of criteria that may be used to prioritise investigations without the need to search entire databases.

¹ Or some other number decided by the investigating authorities

Fig 3: Reference profiles are collected (part of case circumstances or databases) and compared to evidence profiles. A three-step procedure identifies 'matches' which are forwarded to prosecuting authorities for further investigation. Either the candidates are all eliminated, in which case a more relaxed or focused search may be requested, or one or more candidates become suspects and the lab moves from investigative to evaluative mode, in considering the evidence in relation to propositions put forward by the authorities and the case circumstances. Dotted arrow lines are optional.

5.3 Why use a stepwise strategy to extract candidate matches?

The LR method is preferred to the MAC when mixtures are compared to large DNA reference databases [25]. Studies show that a quantitative model, such as EFM, outperforms the qualitative model [26, 33]. However, as illustrated in this paper, the time taken to perform analyses with EFM is a limitation, hence the step-wise approach was introduced. An argument for using MAC as a first step is consistent with many labs that have introduced an upper threshold on the number of mismatches allowed before a result can be reported. For example, if, the analytical procedure accepts a maximum of 4 dropouts, such a rule would be easy to apply in CS with the added benefit of speeding up comparisons (as opposed to not using this threshold).

CS makes use of the qualitative model since it is very fast and performs well (also implemented in SmartRank [18]). We have implemented the MLE approach for improved performance [26]. For the Fusion 6C validation study we found that this approach performed well by giving a high LR for contributors and a low LR for non-contributors (except for the high dropout samples where the number of contributors were underestimated). However, as illustrated for the real case example, this model can sometimes give misleading LR values since it does not use the peak height model – this would typically happen when searching large DNA databases or considering relatives, as illustrated in this paper and by others [26]. Consequently, we recommend that the three-step strategy is normally applied (Fig 3). This paper (along with a previous study [21]) shows that this is accomplished within a reasonable time period.

5.4 The performance of searching large national DNA databases

In order to test the limitations of CS, we tried different sizes of reference databases which were based on 24 loci (also included non-autosomal markers). For 1 mill. generated references, in addition to the profiles in the real case example, the memory required was 13 GB. It was not possible to carry out computations with an 8 GB platform. Once all the data were imported in CS, the total time taken for CS to compare and visualize the results of comparing more than 1 mill. references against 44 mixtures was 35 min + “additional”. Here, “additional” refers to the strategy used after the MAC comparison. As a result, we found that the qualitative/quantitative based methods were important to apply in order to reduce the very high number of false positives: The MAC=0.8 threshold returned 56,445 (generated) candidates, where the higher order mixture sample **04.01** constituted 44,397 of these. Sequentially using the qualitative method with threshold LR=1000 recovered 229 (generated) candidates. Last, when the quantitative model was applied, an LR threshold of 1000 was suitable to obtain a manageable list of 140/160 (generated) candidates (maxK=3/4). However, increasing it to 1 mill removed almost all (generated) references from the match list, except of one instance which had $\log_{10}LR=6.1$. This observation is entirely in keeping with theory by Gill et al. (2014) [28] who stated: “How often a $LR=x$ or more occurs is accommodated by $P(LR > x|Hd) < 1/x$, so we never expect more than N/x chance matches, where N is the size of the database. The number of non-contributors with a greater LR than x , in a database of size N , is $N \cdot P(LR > x|Hd)$ and it is crucially dependant on N .” Hence searching against a large number of reference samples with low LRs as thresholds causes an investigative limitation because of the increase of false positive results. Still, the number of false positives observed in our demonstration were much less than the expected upper boundary from such a search, i.e., ‘number of samples’*‘database size’/‘LR threshold’ [5, 28]. This would be 44,000 with a threshold of LR=1000 and 44 with LR=1e6.

Regarding large DNA databases, CS is very memory demanding. The bottleneck comes down to the visualization of large matrices in the graphical user interface. We recommend using the non-graphical software *dnamatch2* [21] as a memory efficient alternative (it is based on the same methods used in CS). Another alternative is to use EFM or *SmartRank* [18] for searching one evidence profile (also replicates) at a time in very large DNA databases for a specified set of propositions.

5.6 The flexibility of CS

An important aspect of CS is that it is very easy to customise: The user can provide different model settings and search strategy thresholds, and even tailor the type of data that can be imported into the software. CS also has the possibility to create simple HTML reports, with the limitation that it does not directly produce reports in pdf format. Here, the user can select what part of the data or results that should be exported to the report.

CS makes it possible to define a lab-specific “importData” function. This means that there are no rules to describe the format of DNA profiles used as input, so long as the function returns a valid format which is recognizable by CS. This enables a general framework to import data into CS, limited by the R language and its supporting R-packages. This flexibility of approach means that it can be integrated into laboratory systems that have different data-formats to that used by us.

Another possibility, which is currently implemented, would be to use CS to analyze next generation sequencing based data where one may want to consider the “longest uninterrupted sequence (LUS)” format (together with the ForenSeq kit, for instance) as presented in Just and Irwin (2018) [34].

A tutorial for the software is available at <http://www.euroformix.com/casesolver>.

5.7 The validation status of CS

The software is ‘investigative’ rather than ‘evaluative’. The aim is to capture as many true ‘matches’ as possible, minimizing the number of false negatives and false positives (section 4.3). CS is an improvement on standard database screening that relies only on deconvolution and is also an improvement on qualitative screening used by *SmartRank*. Defining the performance of software in terms of false positives and negatives is an important part of validation and provides a benchmark statement of the performance to compare future improvements, i.e., validation is a continuous process. There will be limitations, particularly when the person of interest is a contributor with very low level because the corresponding LR will be low and below threshold (section 4.3). The system is designed to be exploratory and there is flexibility for the user. After ‘matches’ have been found, the scientist enters the evaluative phase of the analysis which forms the basis of the court report – this means that the LR can be recalculated with EFM or any other probabilistic software using specific frequency databases, θ -correction, number of contributors etc. as shown in Fig. 3 and this is covered by separate validation studies reported elsewhere (e.g. [26, 35, 36]).

Supplement D describes program checking carried out using R scripts that are available at the webpage www.euroformix.com/evaluations in a file named ‘NumEval 2.1.0’.

Since November 2017, CS has been actively used in casework at the Department of Forensic Sciences (Oslo University Hospital) in Norway. Here, CS has been integrated into the data system and work flow in order to allow seamless transfer of data. We have found CS to be an important tool to support the findings of the reporting scientists, and to discover manual typing errors or artefacts from an earlier stage in the analysis process. There is a lot of flexibility in the software so that it can be utilized for specific purposes. Consequently, it is necessary for labs to carry out in-house validation but this is true for any software.

5.8 Future improvement

The estimation of the number of contributors was conducted by using the maximum likelihood estimation of the qualitative model with a penalty term, as which was introduced in Bleka et al. [26]. From the examples in the paper, we found some situations where the number of contributors was underestimated, leading to false negatives. We hope to further improve the method in a future update.

The method for relatedness testing in CS was only based on simple allele sharing (IBS). In a future update the LR for hypothesized relationships could also be calculated to further improve the test.

More sophisticated theory can be developed to identify whether contributors in two separate mixtures are the same. By generalizing the traditional methodology it is possible to calculate a LR score for the comparison of proposition “Two mixtures share a common contributor” versus “Two

mixtures do not share any contributors”[37]. Hence this could be applied to make investigative leads about common unknown contributors of complex mixtures that are otherwise difficult to resolve.

6 Conclusion

We have demonstrated that CaseSolver is an efficient graphical expert tool to produce a fast overview in forensic cases with many single source and references profiles together with complicated mixtures. It is ideal when there are many pairwise comparisons needed (typical for screening large databases). This shows that applying such tool in DNA-labs would dramatically improve the efficiency of profile comparisons. However, it is important to separate the investigative from the evaluative process of interpretation – CS is an investigative tool used to support the reporting scientists, to discover potential suspects whom may be put forward for evaluative purposes. Evaluation can be carried out with respect to any probabilistic software (i.e. it is not limited to EuroForMix).

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8 Conflict of interest

The authors declare that they have no conflict of interest.

9 Appendix

Supplementary material

10 References

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