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Research Paper

Facilitating complex DNA mixture interpretation by sequencing highly polymorphic haplotypes

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ABSTRACT

Interpretation of complex DNA mixtures is an ongoing challenge in the field of forensic genetics. Commonly used STR markers are quite polymorphic, enabling very high statistical association between a single source DNA profile from a crime scene and a matching suspect. STR typing of low order mixtures with two and three contributors also commonly produces high statistical association for a contributor, using current interpretation software. However higher order mixtures, with four contributors or more, are more challenging. Shared alleles among the many contributors may complicate the correct assessment of the number of contributors to a mixture and decreases the statistical support for inclusion of contributors. Recently, there is a rising use of massively parallel sequencing for forensic applications, and markers such as SNPs and short haplotypes are receiving more attention. However, these markers are even less polymorphic than autosomal STRs and are not suitable for complex mixture interpretation. We propose to use a different panel of haplotype markers, which contain many SNPs and are very polymorphic. These markers can be sequenced either by standard sequencing technologies or by a new instrument called MinION that sequences DNA as it passes through a nanopore. This instrument is very small and can sequence long stretches of DNA, but suffers from high error rate. We present a method for calling haplotype alleles facing high error rate, and use simulation to test its robustness. We also calculate likelihood ratio (*LR*) between propositions of contribution and non-contribution for individuals that do, and do not, contribute to various complex DNA mixtures. Our results indicate that the correct alleles can be identified in a mixture, despite the high sequencing error rate, and that contributors get high *LR* ($> 10^9$) even in complex mixtures with up to five contributors. Non-contributors receive a very small *LR*, below 1 in most cases (> 98%), which support their exclusion as possible donors to the complex DNA mixtures.

1. Introduction

For many years the interpretation of complex DNA mixtures has posed a challenge for the forensic community. Part of the difficulty stems from the relatively low level of polymorphism of commonly used short tandem repeat (STR) markers. In current commercial STR amplification kits, PowerPlex® Fusion and GlobalFiler®, for over 75% of STR markers, the three most common alleles together account for more than 50% of allele frequencies in the population [1]. In complex mixtures, this translates to many alleles being shared among contributors, making the estimation of the number of contributors difficult. The presence of many common alleles in the mixture also reduces the strength of the statistical inference of contribution. One approach to deal with this difficulty is to further increase the number of markers [2,3] or use the highly parallel single nucleotide polymorphism (SNP) typing by microarrays [4]. Another approach is to use more polymorphic markers that reduce sharing of alleles and increase the power of discrimination [5]. In recent years there has been an increased interest in short SNP-based haplotypes as an alternative forensic marker [6]. The haplotypes proposed were up to 200 bp long and included between 2 and 4 SNPs [7]. Similar to STRs, these markers had alleles with frequencies in the range > 10%, providing little advantage for complex mixture interpretation.

We propose to use more polymorphic haplotypes, which contain more than 10 SNPs. Current massively parallel sequencing (MPS) platforms such as IonTorrent and MiSeq are suitable for genotyping such haplotypes in a single read. Another potential technology for sequencing haplotypes is the emerging nanopore sequencing [8]. The nanopore-based MinION instrument is a tiny sequencer that has a very small footprint and requires only a computer with USB connection to

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operate. It sequences single molecules of DNA as they pass through a pore, without synthesis, which makes the process faster and avoids the decline of accuracy with increasing read length. Reads of tens of thousands of bases are routinely reported [9]. The main drawback of this instrument is the high error rate of the single read. The substitution error rate of MinION is about 5% [10] which is substantially higher than in current MPS instruments such as IonTorrent or MiSeq which have error rates of about 0.1% [11]. The nanopore technology is still under development and improvements in both throughput and accuracy are expected in the near future. In this work we present a method to identify the haplotypes present in a DNA mixture either by standard MPS instruments or the MinION instrument. Simulations are conducted to verify the robustness of this method under various conditions. The efficiency of the method is tested by computing likelihood ratio (*LR*) for true contributors and non-contributors in simulated complex mixtures.

2. Methods and results

2.1. Selecting the loci

The microhaplotype loci proposed by Kidd et al. [7] have a relatively small set of alleles, with high frequencies. We aimed to find better candidates for forensic haplotype loci, and searched for the most polymorphic haplotype loci found in the genome. We have scanned the whole genome sequences of 2504 individuals published by the 1000Genome project [12]. In the first step, the desired size of the haplotypes was selected (either 200 bp or 350 bp) and then the collection of genomes was scanned with a sliding interval of the selected length. For each position, all existing haplotypes were identified and the frequency of each haplotype was estimated. Only SNPs were considered to constitute the haplotypes, ignoring insertions, deletions and larger variations.

Since we searched for the most polymorphic markers, the selection criterion was the lowest homozygosity calculated as Σpi^2 where p_i is the frequency of allele i and the summation is over all the existing alleles in the locus. The homozygosity can be translated into its reciprocal, the effective number of alleles (A_e) [7]. DNA markers with high A_e will have high power of individualization and can help to deconvolute complex mixtures. A_e was calculated for the entire collection of genomes ("global A_e") and for two separate populations, individuals from European descent ("EUR A_e") and individuals of Sub-Saharan Africa descent ("AFR A_e"). We avoided loci positioned within 5 Mbp of centromeres or in chromosomal duplications (> 1000 bp with 90% similarity). Table 1 presents the most informative (high A_e) haplotypes loci discovered by the genome collation scan. Five of the loci are long, between 320bp–350 bp and five are short, below 200 bp. The longer loci are more polymorphic but are also more susceptible to DNA degradation. The actual amplicons of these loci would be somewhat longer in order to accommodate PCR primers. Most of the loci listed in Table 1 are more polymorphic than the most polymorphic STR marker, SE33, that has $A_e = 18$. All the loci in the table are more polymorphic than the median A_e of common STRs at 4.9 [13].

Table 2
The ratios of contributors in the simulated mixtures. Mixtures containing between two and five contributors with equal or variable contribution ratios were examined.

Mixture type	Ind1	Ind2	Ind3	Ind4	Ind5
Mix1	0.5	0.5			
Mix2	0.33	0.33	0.33		
Mix3	0.25	0.25	0.25	0.25	
Mix4	0.2	0.2	0.2	0.2	0.2
Mix5	0.1	0.9			
Mix6	0.1	0.2	0.7		
Mix7	0.1	0.2	0.3	0.4	
Mix8	0.1	0.15	0.2	0.25	0.3
Mix9	0.05	0.15	0.25	0.55	

2.2. Case simulations

Sequence reads from DNA mixtures were simulated to resemble the reads expected to be generated by the MinION instrument for PCR products of the 10 loci described in Table 1. First, individuals were simulated with haplotypes randomly sampled from the population according to the European haplotype frequencies found in the 1000Genome project for each locus. Then, a mixture was created with a predetermined number of individuals and contribution ratios. Table 2 lists the types of mixture and ratios used. Mixtures containing between two and five contributors were tested. Mix1-Mix4 represent equal DNA contribution of all contributors, while Mix5-Mix9 represent mixtures with variable DNA contributions. Next, a random set of typed DNA strands was simulated by sampling from the pool of haplotypes in the mixture according to their relative ratios. The random sampling imitates the stochastic nature of strand selection when pipetting an aliquot from a low-concentration DNA dilution. In the last step, the DNA strands were transformed to simulated sequencing reads by randomly introducing errors according to the average substitution rates of the MinION instrument per source nucleotide and called nucleotide (Supplementary Fig. 8 by Jain et al. [10]). Various depths of coverage in the range of 50–500 were examined. Depth of coverage is the number of reads produced in the sequencing reaction that cover a specific genomic locus. The depths of coverage we examined for MinION are relatively low for a massively parallel sequencing due to the lower overall throughput of MinION sequencing.

Each simulated mixture was compared to two suspects. One suspect is a true contributor and the other is a random individual. All contributors in the mixture, except the single tested suspect, were assumed to be unknown and unrelated individuals.

Table 1
Potential loci with many SNPs in a short haplotype displaying high level of polymorphism. The chromosomal positions are according to the human genome reference GRCh37. A_e is the effective number of alleles. EUR stands for European decent and AFR stands for Sub-Saharan Africa descent.

Chromosome	Chromosomal start position	Length (bp)	Number of SNPs	Number of haplotypes	Homozygosity	Most common allele frequency	A_e		
							Global	*EUR*	*AFR*
3	4609154	320	23	357	1.5%	4.4%	64.9	55.1	44.7
9	129479273	342	47	417	1.8%	4.9%	55.0	33.0	30.3
10	16716549	324	26	352	2.1%	5.7%	47.8	31.0	37.6
20	5646531	349	45	391	2.4%	8.6%	41.0	36.4	25.4
2	174285315	324	55	241	2.9%	7.3%	34.6	18.6	31.5
3	11955875	197	16	306	2.4%	6.1%	41.3	18.6	19.2
3	5825196	197	44	421	3.3%	8.6%	29.9	19.6	11.9
2	28790522	189	18	98	4.3%	10.2%	23.5	10.4	23.0
6	33024132	192	15	99	5.0%	11.1%	19.9	10.1	14.0
13	70865992	193	19	85	6.8%	17.4%	14.7	12.0	10.4

2.3. Calling the alleles

The haplotypes we propose are complex and contain many SNPs. The high per-SNP error rate (~5%) of the MinION instrument accumulates throughout the haplotype resulting in most reads containing at least one mistake. This makes haplotype calling by simple consensus or counting impractical. The strategy we used to call haplotypes was a sequential partition of the reads into two groups, based on a single SNP each time. The SNP by which the group is divided is the SNP with the lowest probability of being the result of random errors. After each partition into two groups, each group is then again divided into two, and so on. The partition process continues until no group of reads has a SNP with a probability less than X of being the result of random errors. X is a laboratory-defined partition threshold (e.g. 0.01).

We assume that errors in the reads of an SNP are random events that are binomially distributed. The probability of an error is dependent on the source nucleotide, the erroneous nucleotide and the surrounding sequence. In this work we used the error rates considering the source and erroneous alleles according to the published substitution rates [10]. A more exact probability of error can be generated by running known samples and empirically measuring the frequency of substitution for each SNP individually.

To select the SNP by which the group of reads will be partitioned, we look for the SNP for which the number of reference alleles and alternative alleles can least likely be explained by only one allele being truly present while the other allele emerging from errors. We denote by M the number of reads with the less frequent allele, and by T the total number of reads. Let S denote the substitution rate from the more frequent allele to the less frequent allele, and let E denote the number of errors. Assuming the errors are random and independent, $E \sim \mathrm{Bin}(T, S)$. We calculate $\Pr(E \geq M)$for every SNP in the haplotype and find the SNP with the smallest probability of error. If all SNPs in the group of haplotypes have $\Pr(E \geq M)$ higher than the partition threshold, then the partition of this group stops.

After the partitions of all groups and sub-groups are completed, a consensus sequence for each final group is generated and the intensity of each consensus sequence is recorded as the number of reads in the group. A detection threshold of minimum read count (e.g. 10) is used to eliminate haplotypes with low number of reads. A second threshold is set to eliminate probable false calls or artifacts, similar to a stutter threshold for STR markers. If a low intensity haplotype in the set differs from a high intensity haplotype by a single substitution and is below a relative intensity threshold (e.g. 20%) of the high haplotype, the low intensity haplotype is deemed to be an artifact and eliminated from the set of called haplotypes.

The consensus sequences of all groups that passed the detection and artifact thresholds are considered as the final set of detected haplotypes in the mixture. The frequencies of the detected haplotypes are derived from a population sample of typed individuals. For a rare allele or a new allele that was not typed in the population sample, a minimum frequency of 5/2N is applied according to the recommendation in the NRCII report [14], where N is the number of individuals in the population sample.

2.4. Establishing thresholds

The thresholds used in allele calling are required to balance between the two errors associated with allele calling known as drop-in and drop-out. Drop-out is the failure to detect in a mixture an allele that exists in one of the contributors to the mixture. The lower the contributors' ratio in the mixture, the higher the probability that his alleles will drop-out. Drop-in is the identification of an allele in a mixture although it does not originate from any of the contributors to the mixture. Generally, the aim is to set thresholds that would keep drop-in at a minimal level while retaining as many real alleles as possible, i.e. avoiding drop-out. Fig. 1 show the drop-in and drop-out frequencies under various threshold levels. The data in Fig. 1 are the aggregation of all drop-in and drop-out events in all simulated mixtures types described in Table 2. Each mixture type was simulated 100 times under each combination of threshold parameters.

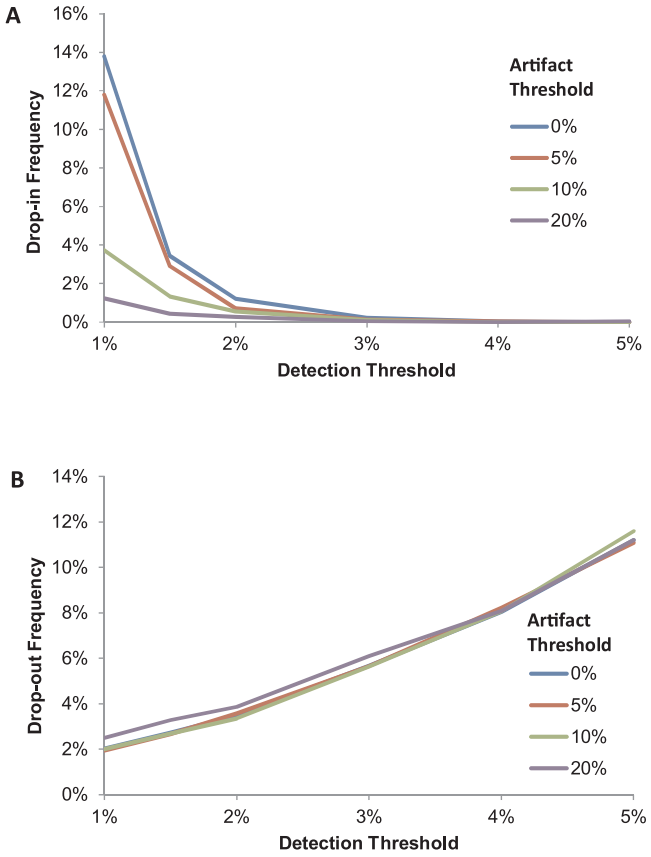

Fig. 1. Error rates as a function of the detection threshold. Mixtures with five coverage depths [100, 150, 200, 300, 500] are included. The *X*-axis values are the percentage of total coverage a haplotype count must pass to be called as present. The four lines represent different values of the artifact threshold (0%, 5%, 10%, 20%). Figures **A** and **B** show the drop-in (type I error) and Drop-out (type II error) frequencies, respectively.

In the following calculations of likelihood ratio we are using detection threshold of 2% of overall coverage and artifact threshold of 20% since these parameters kept drop-in at very low level below 0.003 while drop-out occurred in less than 0.04 of alleles. These low drop-in and drop-out rates indicate that the partition method combined with appropriate thresholds is a robust way to call haplotype alleles in face of high sequencing error rates. The partition threshold was evaluated for a range between 0.1 and 0.001 but had insignificant influence on drop-in and drop-out levels (data not shown). In further analysis this threshold is set to 0.01.

2.5. Likelihood ratios

Likelihood ratios (*LR*) were calculated according to the semi-continuous split-drop model [15]. In this model, the intensity of alleles is not taken into account, but drop-in and drop-out of alleles are considered. The “split-drop” refers to the individual probabilities of drop-out assigned to the suspects' alleles and to the unknown contributors' alleles. The drop-out probabilities are maximum likelihood estimates (MLE), numerically maximized for each proposition. Drop-in rate was set to 0.01. For each case-mixture, the propositions were:

Prosecution hypothesis (H_p): the suspect and N-1 unknown individuals are contributing to the mixture.

Defense hypothesis (H_d): N unknown individuals are contributing to

the mixture.

In this study the true known number of contributors was used for *N*. For real forensic case-work samples, the number of contributors (*N*) can be estimated according to the least number of contributors required to explain all the alleles detected in the mixture. For example, if the locus with the most alleles in the mixture has 7 or 8 alleles then *N* is estimated to be 4 contributors. While this approach performs poorly for STR typing of high order mixtures [16], our loci are much more polymorphic, allowing correct estimation of *N* in most cases (> 95%) even for five-contributor mixtures (Fig. S1).

In order to test the robustness of the method in two aspects, both to include true contributors and to exclude non-contributors, each simulated mixture was compared separately to two suspects. One time the suspect was one of the contributors to the mixture (Ind1 in Table 2) and the second time the suspect was a random unrelated individual. All individuals were simulated according the European haplotype frequencies, and the *LR* was calculated using European frequencies as well. In Supplementary material section "Implications of unknown ancestry" we present results for mixtures containing individuals from both European and Sub-Saharan Africa ancestry, calculated with haplotype frequencies of either population.

Fig. 2 shows the average *LR* for actual contributors in various mixtures. As expected, the higher the coverage, the higher the *LR* for true contributors. Comparing Fig. 2A and B demonstrates that the number of individuals in the mixture has a relatively smaller impact on *LR* while the proportion of the individual in the mixture has the main impact. Contributors of 10% of DNA in mixtures of various complexity

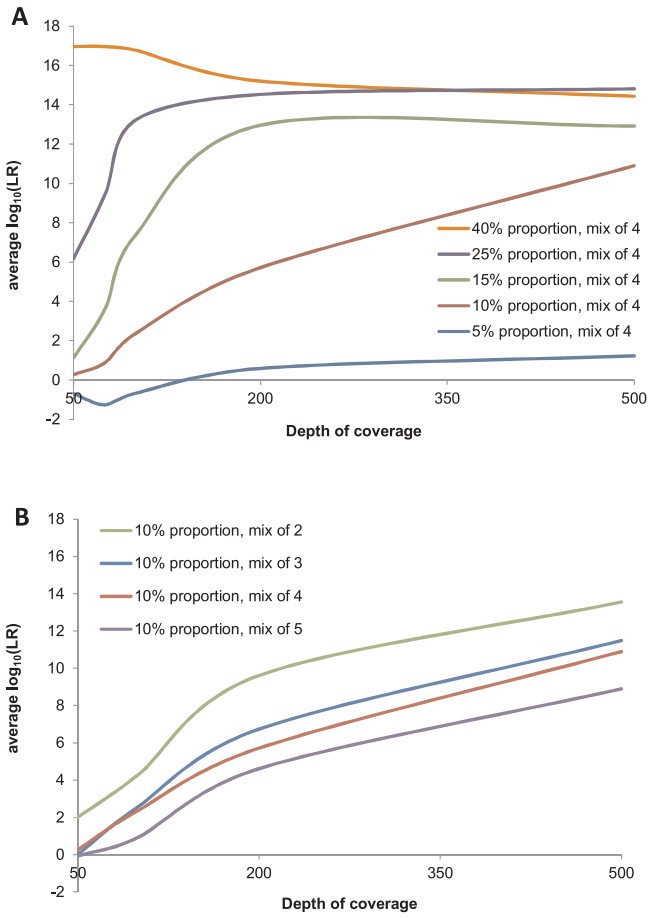

Fig. 2. Likelihood ratio of contributors to mixtures as a function of depth of coverage. Each line represents a different scenario of mixture complexity and contributors' DNA proportion in the mixture. **A.** LR of contributors of various proportions to a four-person mixture. **B.** LR of a 10% contributor to mixtures of various numbers of contributors.

from three to five individuals all had similar *LR*. On the other hand contributors of various DNA proportions to a four person mixture had very different LR results. A contributor of 25% of the total DNA in the mixture had a high *LR* (10^9 on average) even at 75 depth of coverage, while a contributor of 10% required depth of coverage of about 500 to reach similar *LR*. Very minor contributors of 5% of the total DNA in a mixture received a low *LR* (17 on average) even with coverage of 500.

Fig. 3 shows the Tippett plot [17], a cumulative distribution of *LR* among contributors and non-contributors. These results encompass more than 11000 simulated mixtures, including mixture types 1–9 of Table 2 and five coverage depths [100, 150, 200, 300, 500]. Non-contributors get mostly very small *LR*, that support their exclusion from the mixture, with only 2% getting an *LR* above 1 and none getting *LR* above 300. About 99% of true contributors had *LR* above 1, and 88% had *LR* above 10^6, providing very strong support for their inclusion in the mixtures. These results indicate that the method is robust and can accurately assign contribution to complex mixtures, despite using only 10 loci, low depth of coverage and high error rate.

Fig. 4 demonstrates the impact of the level of polymorphism, measured as A_e, on the ability to robustly associate contributors to complex DNA mixtures. For this analysis we used simulated sets of loci with varying number of markers and varying levels of A_e. The loci were simulated to resemble those in Table 1 in terms of length and numbers of SNPs, except the frequencies of all haplotypes in a locus were equal. For example a set might contain 10 loci, each one with 20 haplotypes, and each haplotype having a frequency of 0.05. This corresponds to a set of 10 loci with A_e of 20. The simulation of sequencing was done as previously with error rates typical to the MinION instrument. The depth of coverage was 200. As can be seen in Fig. 4, using loci with A_e of 5 requires about 50 loci to achieve high *LR* ($> 10^9$) for inclusion in a mixture of four contributors. Increasing the A_e of the loci to 15 greatly reduces the number of required loci to approximately 12. An increase in A_e beyond 20 has a much smaller effect. This indicates that for complex mixture interpretation loci with A_e of at least 15 are preferable.

3. Discussion

Short haplotypes are a powerful type of forensic DNA marker that can be typed by massively parallel sequencing [18]. The MinION instrument is a new potential technology for sequencing loci that contain many SNPs and complex haplotypes. We have presented a method for identifying haplotypes present in a DNA mixture despite high error rates that are associated with MinION sequencing. The method employs sequential partition of the sequencing reads into groups representing the haplotypes present in the mixture. Paired with optimized allele calling thresholds, the method keeps drop-in and drop-out errors at a low level suitable for forensic interpretation. We further demonstrated through simulations that the sequencing results provide a robust means for inclusion or exclusion of contributors and non-contributors respectively, based upon likelihood ratio calculations. We also present a method for identifying loci with very polymorphic haplotypes ($A_e > 15$) and use a subset of such loci for the simulation analysis.

Loci with high level of polymorphism of $A_e > 15$ have much higher value in analysis of complex DNA mixtures in comparison to loci with $A_e < 5$ as were proposed previously [5]. A set of 10 highly polymorphic loci as described in Table 1 can correctly include a contributor to a mixture of 4 or 5 contributors with a high *LR*, while less polymorphic loci would require the use of many tens of loci.

The described method can easily be applied to sequences generated by other technologies such as MiSeq or Ion-Torrent. Because these technologies have lower sequencing error rate, the calling thresholds would be lower and a higher sensitivity to minor contributors can be achieved. On the other hand, the MinION instrument has many advantages such as its small size and low infrastructure requirements, giving it the potential to be implemented in smaller and more remote labs.

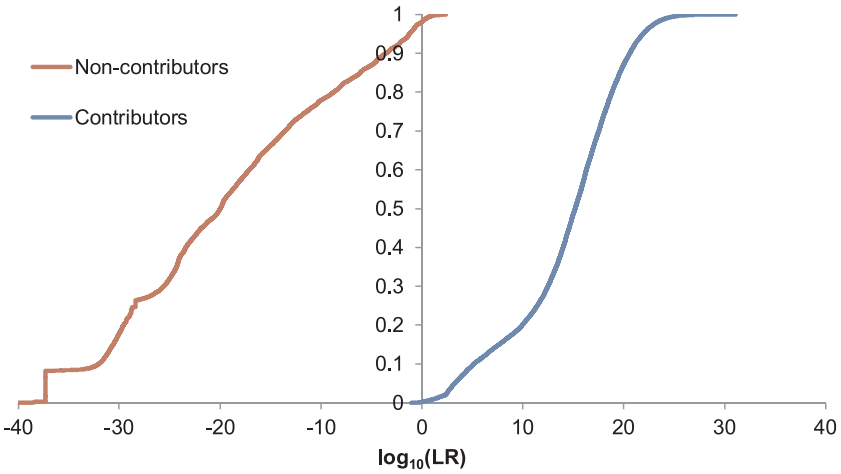

Fig. 3. Tippett Plot. Cumulative distribution of $\log_{10}$(likelihood ratio) of contributors (blue line) and non-contributors (red line). The distribution includes mixture types 1 to 9 described in Table 2, and coverage depths of 100, 150, 200, 300 and 500.

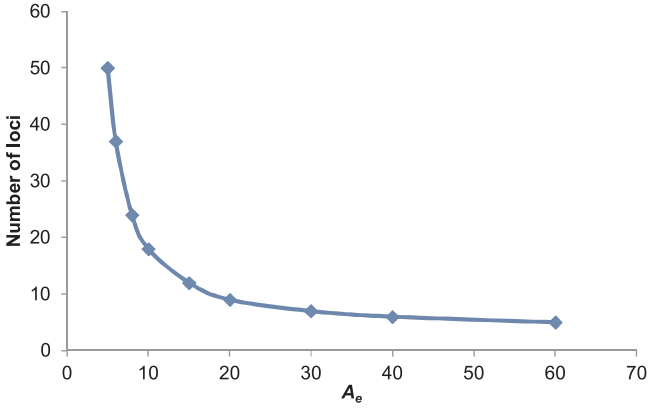

Fig. 4. Number of Loci required to achieve an average LR above 10^9 for a contributor in a mixture of four contributors as a function of A_e. Sequencing coverage was 200 and the mixture contained four individuals with equimolar contribution.

Since the loci we describe were found based on a single public database, experiments are underway to test these loci by deep massively parallel sequencing and prove their true level of polymorphism.

4. Conclusion

Highly polymorphic short haplotypes, typed by massively parallel sequencing, can provide an excellent tool for interpretation of complex DNA mixtures. Simulations indicate that complex mixtures can be accurately interpreted even under constraints of low coverage and high error rates characteristic of the upcoming nanopore sequencing technology.

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.2018.05.001.

References

[1] T.R. Moretti, L.I. Moreno, J.B. Smerick, M.L. Pignone, R. Hizon, J.S. Buckleton, et al., Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States, Forensic Sci. Int. Genet. 25 (2016) 175–181.
[2] D.Y. Wang, S. Gopinath, R.E. Lagace, W. Norona, L.K. Hennessy, M.L. Short, et al., Developmental validation of the GlobalFiler((R)) Express PCR Amplification Kit: a 6-dye multiplex assay for the direct amplification of reference samples, Forensic Sci. Int. Genet. 19 (2015) 148–155.
[3] M.G. Ensenberger, K.A. Lenz, L.K. Matthies, G.M. Hadinoto, J.E. Schienman, A.J. Przech, et al., Developmental validation of the PowerPlex((R)) Fusion 6C System, Forensic Sci. Int. Genet. 21 (2016) 134–144.
[4] L. Voskoboinik, S.B. Ayers, A.K. LeFebvre, A. Darvasi, SNP-microarrays can accurately identify the presence of an individual in complex forensic DNA mixtures, Forensic Sci. Int. Genet. 16 (2015) 208–215.
[5] K.B. Gettings, K.M. Kiesler, S.A. Faith, E. Montano, C.H. Baker, B.A. Young, et al., Sequence variation of 22 autosomal STR loci detected by next generation sequencing, Forensic Sci. Int. Genet. 21 (2016) 15–21.
[6] K.K. Kidd, A.J. Pakstis, W.C. Speed, R. Lagace, J. Chang, S. Wootton, et al., Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics, Forensic Sci. Int. Genet. 12 (2014) 215–224.
[7] K.K. Kidd, W.C. Speed, Criteria for selecting microhaplotypes: mixture detection and deconvolution, Investig. Genet. 6 (2015) 1.
[8] R.H. Nanda, Y. Liu, T.W. Gillespie, J.L. Mikell, S.S. Ramalingam, F.G. Fernandez, et al., Stereotactic body radiation therapy versus no treatment for early stage non-small cell lung cancer in medically inoperable elderly patients: a National Cancer Data Base analysis, Cancer 121 (2015) 4222–4230.
[9] M.A. Madoui, S. Engelen, C. Cruaud, C. Belser, L. Bertrand, A. Alberti, et al., Genome assembly using Nanopore-guided long and error-free DNA reads, BMC Genomics 16 (2015) 327.
[10] M. Jain, I.T. Fiddes, K.H. Miga, H.E. Olsen, B. Paten, M. Akeson, Improved data analysis for the MinION nanopore sequencer, Nat. Methods 12 (2015) 351–356.
[11] S.J. Salipante, T. Kawashima, C. Rosenthal, D.R. Hoogestraat, L.A. Cummings, D.J. Sengupta, et al., Performance comparison of Illumina and ion torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling, Appl. Environ. Microbiol. 80 (2014) 7583–7591.
[12] A. Auton, L.D. Brooks, R.M. Durbin, E.P. Garrison, H.M. Kang, J.O. Korbel, et al., A global reference for human genetic variation, Nature 526 (2015) 68–74.
[13] C.R. Hill, D.L. Duewer, M.C. Kline, M.D. Coble, J.M. Butler, U.S. population data for 29 autosomal STR loci, Forensic Sci. Int. Genet. 7 (2013) e82–83.
[14] N.R. Council, The Evaluation of Forensic DNA Evidence, N.R. Council, Washington, DC, 1996.
[15] H. Haned, K. Slooten, P. Gill, Exploratory data analysis for the interpretation of low template DNA mixtures, Forensic Sci. Int. Genet. 6 (2012) 762–774.
[16] H. Haned, L. Pene, J.R. Lobry, A.B. Dufour, D. Pontier, Estimating the number of contributors to forensic DNA mixtures: does maximum likelihood perform better than maximum allele count? J. Forensic Sci. 56 (2011) 23–28.
[17] P. Gill, J. Curran, C. Neumann, A. Kirkham, T. Clayton, J. Whitaker, et al., Interpretation of complex DNA profiles using empirical models and a method to measure their robustness, Forensic Sci. Int. Genet. 2 (2008) 91–103.
[18] K.K. Kidd, W.C. Speed, A.J. Pakstis, D.S. Podini, R. Lagace, J. Chang, et al., Evaluating 130 microhaplotypes across a global set of 83 populations, Forensic Sci. Int. Genet. 29 (2017) 29–37.