Computational software for assessing allelic dropout

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ABSTRACT

Allelic dropout is a failed amplification of an allele which usually happens when the concentration of the DNA sample is low. As there is a missing genotype, the result of the DNA profiling will significantly be affected. One way to overcome this problem is by using computational software that considers the dropout event within its algorithm. This review is aimed to discuss several software that have been created to serve this purpose. All of the listed software turn to implement Maximum Likelihood (LR) algorithm within their calculation; however, they use different parameters and variables. This review showed that allelic dropout should not be evaluated alone; it correlates with other events in creating a low quality of DNA. Therefore, a comprehensive algorithm that consider all of the factors should be built to best estimates the allelic dropout rate within a data.

Keywords: software; allele; dropout; forensic; profiling

INTRODUCTION

The invention of forensic DNA profiling methods, such as DNA fingerprinting, has provided a huge contribution in criminal investigation. Previous study showed that DNA fingerprinting via Southern blot hybridization produced unique DNA fragments which correspond to one specific person (Gill, Jeffreys, & Werrett, 1985). However, this approach is highly depended on the quality of the sample, which sometimes may be poor. This review will discuss the general idea behind forensic DNA profiling, its limitation due to poor quality of the sample, and several Bioinformatics tools that have been developed to overcome the limitation.

Deoxyribonucleic Acid (DNA)

DNA, commonly known as deoxyribonucleic acid, is the hereditary material of organisms, particularly humans (GHR, 2018a); it contained all information that is needed to build and maintain an organism (Nature, 2014). The information is coded by four chemical compounds (bases): guanine (G), cytosine (C), adenine (A), and thymine (T). The group of three bases, called codon, will undergo transcription and translation, encoding amino acid. Currently, there are 20 amino acids that are needed by human; each of them is encoded by different order of codon (Figure 1). Then, the amino acids are linked together to form protein, a complex molecule that plays a crucial role in regulation inside the body (GHR, 2018b).
Figure 1. Amino acid table. In RNA molecule, thymine (T) base is transcripted into uracil (U). Phe: phenylalanine; Leu: leucine; Ile: isoleucine; Met: methionine; Val: valine; Ser: serine; Pro: proline; Thr: threonine; Ala: alanine; Tyr: tyrosine; His: histidine; Gln: glutamine; Asn: asparagine; Lys: lysine; Asp: aspartic acid; Glu: glutamic acid; Cys: cysteine; Trp: tryptophan; Arg: arginine; Gly: glycine. Grey: start codon; pink: stop codon(s).

Moreover, each base pairs up with each other, G with C and A with T, known as base pair (bp). The human genome consists of 3.2 million base pairs; however, only 2% of them that encodes protein, while the remaining are considered as junk DNA. This non-coding region contains a highly polymorphic area which is known as mini- or micro-satellites, characterized by a repetitive sequence of DNA. The micro-satellites, also known as short tandem repeats (STRs), are consisted of 2 until 9 repeated base pairs and located in the entire genome, while mini-satellites are consisted of 9 until 100 repeated base pairs and found in specific site of the chromosome (Panneerchelvam & Norazmi, 2003). Because of their high mutation rate, these two regions, particularly micro-satellites, are widely used in DNA profiling method.

**Short-Tandem Repeat (STR) Typing**

Short tandem repeats (STR), also known as microsatellites, are short repeated DNA sequence whose locations are adjacent to each other. It is highly polymorphic and comprises around 3% of human genome (Fan & Chu, 2007). According to Dauber et al. (2003), the mutation rate of 23 different microsatellite loci ranged between $4 \times 10^{-8}$ to $8.3 \times 10^{-5}$; it was several magnitudes higher than human genome mutation rate, which was estimated to be $\sim 1.1 \times 10^{-8}$ (Roach et al., 2010). As a result, STR is commonly used in DNA profiling. In STR typing, we can get a greater discrimination value by using more STR loci, as the chance for two persons to have the same exact repeat units with the analyzed STR becomes much lower (Panneerchelvam & Norazmi, 2003). For example, the DNA profiling is based on five different STR loci; the suspect has alleles that present in 1 percent, 2 percent, 8 percent, 3 percent, and 10 percent of the population, respectively. Under the assumption that each loci is independent, the chance to find the suspect is $0.01 \times 0.02 \times 0.08 \times 0.05 \times 0.1 = 0.00000008$ or 8 in 100 million. Currently, forensic profiling uses 13-17 STR loci to get the best discrimination value between individuals, such as Combined DNA Index System (CODIS) which is utilized in USA and Canada (Bianchi & Lio, 2006).

Furthermore, the microsatellites alleles within each STR locus is codominant (Panneerchelvam & Norazmi, 2003). Every individual has two alleles that are inherited based on Mendelian law: one from the father and one from the mother. Therefore, the alleles can be either heterozygous or homozygous. In heterozygous allele, there will be two bands that represent two alleles with different size, while the homozygous allele will produce one band that represents two alleles with the same length. For example, the father has allele in the first and second loci (heterozygous), while the mother only in the fourth loci (homozygous). Their child will possess allele either in the first and fourth loci, or second and fourth loci.

Even though STR typing works well when dealing with high-quality sample, it may be different when the DNA of the sample has degraded, resulting a poor quality of sample. There are several factors that may affect the quality of the sample, including environmental and chemical degradations (McCord et al., 2011). As the DNA concentration is low, there might be an allele that is failed to be amplified.
during PCR amplification, known as allelic dropout.

Allelic Dropout

Allelic dropout is a situation where one allele is lost during polymerase chain reaction or PCR (Stevens et al., 2017). It may happen when the sample contains a very low amount of DNA (Taberlet et al., 1996), resulting a failed amplification of one or more allele(s). As a result, a heterozygous person may be identified as a homozygous one, or even cannot be identified at all (missing genotype). Even though fatal, allelic dropout is hard to be identified, as the PCR runs successfully but produces an incomplete data. The example of allelic dropout is shown in Figure 2.

![Ladder](image1.png)

**Figure 2.** Example of PCR result with allelic dropout. Both of the father and mother have homozygous allele on the eighth and seventh loci, respectively. However, PCR amplification of the child sample only produces allele in the seventh loci. As the STR allele is codominant, the child’s allele in the eighth loci should have been dropped out.

One approach to overcome this problem is by re-sequencing the sample (Taberlet et al., 1996). However, allelic dropout often happens when the quality of the sample is poor (low DNA concentration) which makes repeated genotyping impracticable. Moreover, it is also costly and often uninformative (Wang, Schroeder, & Rosenberg, 2012). Therefore, computational approach become an alternative to solve this problem.

**COMPUTATIONAL SOFTWARE**

In fact, there is a lot of algorithms that have been developed to assess allelic dropout. Sample algorithm, such as Random Match Probability (RMP) and Combined Probability of Inclusion (CPI), treat this problem by only considering the loci where allelic dropout is unlikely, while the one which may undergo allelic dropout is omitted (Inman et al., 2015). However, this approach has a risk of underestimating the potential of a contributor and/or including the false one. Moreover, there is also Random Man Not Excluded (RMNE) algorithm; similar with the previous algorithm, RMNE does not consider allelic dropout event (i.e. allele is present only when it can be observed and absent otherwise). However, Van Nieuwerburgh, Goetghebeur, Vandewoestyne & Deforce (2008) have proposed the improvement of RMNE algorithm that considers dropout event.

Currently, the most widely-accepted algorithm to solve this problem is likelihood-ratio (LR) algorithm. In LR framework, the ratio of probability of the evidence under two different hypothesis is calculated (Inman et al., 2015); it makes us able to directly compare the probabilities, thus determining the potential contributor. The general equation is:

$$LR = \frac{Probability\ of\ suspect\ to\ be\ the\ perpetrator}{Probability\ of\ other\ person\ to\ be\ the\ perpetrator}$$

However, in LR framework, the number of contributors must be specified, which in some cases may be uncertain. In the following subtopics, several Bioinformatics software that assess this problem, including their parameters, will be discussed.

**MicroDrop**

MicroDrop is a software that estimates allelic dropout rates in nonreplicated STR
genotype data from diploid organism (Wang, 2012); it is written in C++ and executable in both Windows and Linux operating systems. In order to perform its task, MicroDrop uses population-genetic data rather than replicate genotyping, meaning that the number of multiple independent loci from different individuals must be large enough to determine the rates. The software is available at http://rosenberglab.stanford.edu/microdrop.html.

As mentioned before, the allelic dropout may cause missing genotype and false homozygous; it means that there should be positive correlation between number of homozygous person and number of missing data across individual and loci (Wang, 2012). If the primary cause of allelic dropout is sample-related (e.g. low DNA concentration), the correlation will be more significant across individuals; if it is locus-related (e.g. binding affinity between primer and/or polymerase with the DNA), the positive correlation will be significant across loci.

Based on this idea, MicroDrop firstly assess the allelic dropout event by calculating the correlations between number of homozygotes and missing data across individual and loci, respectively, based on Native American microsatellite dataset from University of California (Wang, Schroeder, & Rosenberg, 2012). After that, it estimates the model and its parameters based on five assumptions:

1. All distinct alleles are present at least once in the dataset.
2. All missing and incorrect genotypes are due to allelic dropout.
3. Each copy of allele within a loci has the same probability to be lost.
4. All individuals are unrelated and have the same inbreeding coefficient, meaning that the two allelic copies within a loci of every individual are identical by descent (IBD).
5. Each pair of loci is independent.

There are 3 sets of parameter that are used in this model: allele frequencies (Φ), sample-specific and locus-specific dropout rates (Γ), and inbreeding coefficient (ρ); when ρ = 0, the population is in Hardy-Weinberg equilibrium (HWE). In order to estimate the maximum likelihood (MLEs) of those parameters, expectation-maximization (EM) algorithm is used (Wang, Schroeder, & Rosenberg, 2012). The correlation between the parameters and other variables within the model is shown in Figure 3. In addition, the user can also change the population genetic assumption (inbreeding model or Hardy-Weinberg equilibrium) and dropout factor assumption (only sample-specific, locus-specific, or both factors) according to their dataset.

Figure 3. Graphical representation og MicroDrop model. The arrows indicate the dependency between two vertices. ρ: inbreeding coefficient; Φ: allele frequencies; S: IBD states; G: true genotypes; Γ: sample-specific and locus-specific dropout rates; Z: dropout states; W: observed genotypes. Circle outline: parameters; dashed square outline: latent variables; solid square outline: observed data. The graph was taken from Wang, Schroeder, & Rosenberg (2012).

Table 1. Comparison between real and simulated data of the Native American dataset. r: Pearson’s correlation coefficient; P: p-value. The statistics were taken from Wang, Schroeder, & Rosenberg (2012).

| Parameters        | Real Data | Simulated Data |
|-------------------|-----------|----------------|
| Correlation across individual | r = 0.729, P < 0.0001 | r = 0.900, P < 0.0001 |
| Correlation       | r = 0.099, P = | r = 0.143, P = |
across loci = 0.0341 ± 0.0045

|                | Uncorrected | Corrected |
|----------------|-------------|-----------|
| Heterozygosity | 0.716       | 0.715±0.014 |

According to Table 1, the result of the real data is similar with the simulated data, meaning that the allelic dropout event is well-estimated under the model. The model also successfully corrected the bias in heterozygosity estimation with very low standard deviation. However, it must be taken into account that the model is constructed based on the Native American dataset (i.e. the simulated data is the same with the real data); its performance might be altered when the heterozygosity mechanism of our dataset is different with the Native American one.

**Lab Retriever**

Lab Retriever is a software that automatically calculates the LR by considering allelic dropout event in the calculation (Inman et al., 2015). It derived from the R-code that was proposed by Balding & Buckleton (2009), and improved by GUI addition and running time reduction. There are also several parameters that are changed from the orginal algorithm, which will be discussed in the next part. The executable binaries are executable both in Mac OS X and Windows, and available at https://github.com/SCIEG/LabRetriever.

The general framework of Lab Retriever assumes two hypotheses: H₀ (the suspect is the source of the evidence profile) and H₁ (a random person is the source of the evidence profile). Lab Retriever will calculate the LR based on the following equation:

\[ LR = \frac{P(E|s)}{\sum_{j} P(E|j)P(j)} \]

The numerator is the probability of H₀, while the denominator is the probability of H₁. In calculating P(E|s), the program will consider the allelic drop-in and dropout events that will convert the suspect (s) profile into the evidence (E) profile; this also applies for calculating P(E|j), where j refers to certain specific genotype. On the other hand, P(j) is the probability to find genotype j within a population; it is calculated by using dynamic programming algorithm instead of iteration to reduce the time complexity of the program.

Moreover, there are four parameters that are used within the model: P(DI), P(DO), coancestry adjustment (θ or Fₛ), and Race. The P(DI) and P(DO) refer to the probabilities of an allele to drop-in and dropout, respectively. In this case, P(DI) is used to take a laboratory contamination into account; the default value is set to be 0.01, but the user can change it based on certain laboratory situation (Inman et al., 2015). On the other hand, θ is used to consider the possibility of the suspect to be distantly-related with the real contributor in population level, while Race includes the Caucasian, African-American, and Hispanic population data which derived from the National Institutes of Standards and Technology (NIST). The user can also consider the relationship between the suspect and a relative by accounting the number of IBD between them (Inman et al., 2015).

The initial input screen of MicroDrop is shown in Figure 4. Different with MicroDrop, Lab Retriever does not estimate the allelic dropout rate; instead, it calculates the likeliness of an individual to be the perpetrator based on the inputted probability. In other words, the user needs to estimate the allelic dropout probability of their data. One solution to fulfill this condition is by conducting several tests with different P(DO) and takes the best result. Based on this idea, it may be said that Lab Retriever works complementary with MicroDrop (i.e. estimated rate of allelic dropout from MicroDrop is used as the parameter in Lab Retriever). However, as the assumptions between the two software may differ, further
study is needed to assess the potential collaboration between these two software.

![Figure 4](image.png)

**Figure 4.** The initial screen of MicroDrop. The user can set the parameters of the analysis on the left part of the windows, including the IBD probability. The default value of $P(DI)$ and $\theta$ are both 0.01. The program is available at [https://scieg.org/lab-retriever/](https://scieg.org/lab-retriever/).

**Pedant**

Pedant is a program that estimates the allelic dropout and false allele error rates from STR data in the absence of reference genotype; it is written in Delphi 7.0 and available only in Windows operating system. In order to perform its task, Pedant uses maximum-likelihood method (Johnson & Haydon, 2007); it compares duplicate genotypes and estimates the error rates based on the nature and number of mismatches. The program can be downloaded as a ZIP file at [https://sites.google.com/site/pcdjohnson/home/pedant](https://sites.google.com/site/pcdjohnson/home/pedant).

Different with allelic dropout, false allele is *misgenotyped* instead of failed to be amplified because of several factors, e.g. human error in recording the data (Johnson & Haydon, 2007). These two events need to be differentiated as they differently contribute to the analysis. For example, the genotype of the evidence is AB; however, the suspects have CC and CD genotypes, respectively. According to Broquet & Petit (2004), the occurrence of allelic dropout is generally higher than false allele. In this case, the genotype of the profile (AB) and one of the suspect (CD) should not undergo allelic dropout, meaning that the CC suspect is likely to be the contributor due to the allelic dropout event. In Pedant, the rates of these events are calculated based on five assumptions:

1. Every genotype is diploid and codominant.
2. The population is in HWE.
3. Each sample has the same allelic dropout and false allele probabilities.
4. Each heterozygous allele has the same probability to drop out.
5. A false allele can only occur once in every duplication, e.g. if genotype AA is duplicated into AA and BB, the appearance of genotype B is the combination of one allelic dropout and one false allele events instead of two false allele events.

According to Johnson & Haydon (2007), there are seven possible outcomes in genotype duplication: AA.AA (normal homozygote), AB.AB (normal heterozygote), AA.AB, AA.BB, AB.AC, AB.CC, and AB.CD (the outcome of two allelic dropout event is not included as it can be considered as PCR failure); the expected frequency of each outcome is calculated based on three parameters: the estimated error rates, the probability of allelic dropout ($\varepsilon_1$) and false allele ($\varepsilon_2$), and expected heterozygosity ($H_e$). In this model, false allele is assumed to always precede allelic dropout, producing a homozygous observed genotype. For example, an AA genotype is recorded as AB because of false allele. Then, it undergoes allelic dropout and becomes AA (homozygous). Even though this model does not perfectly fit the reality, it simplifies the expected frequency calculation of the seven outcomes (Johnson & Haydon, 2007). The maximum likelihood of $\varepsilon_1$ and $\varepsilon_2$ are then estimated by using relative log-likelihood function, considering the frequency of each outcome, and plotted in a graph.

The initial input screen of Pedant is shown in Figure 5. Similar with MicroDrop, Pedant estimates the allelic dropout rate of every locus within the dataset. The difference between the two is that MicroDrop automatically calculates the value of $H_e$ for each loci before estimating the dropout rate, while the $H_e$ calculation in Pedant is done separately with the dropout rate estimation. Moreover, Pedant also considers...
the false allele rate, while MicroDrop does not. As they serve the same function but with different model, further study are needed to assess the accuracy of these two.

**Figure 5.** The initial screen of Pendant. The user can plot the opened data by clicking on one of the `plot` tab and click “Plot CIs and ML Search” button.

### LikeLTD

LikeLTD (Likelihoods for Low Template DNA profiles) is an R package that computes the likelihood of several suspects to contribute their DNA to the DNA profile (Balding, 2013); it can be installed by typing `install.packages(“likeLTD”)` in R environment. The newest version is v6.3, whose guide can be retrieved from https://cran.r-project.org/web/packages/likeLTD/likeLTD.pdf. In order to perform this task, likeLTD assesses the presence of suspect’s DNA within the crime scene profile (SCP); this information are gathered from every locus to determine which suspect who is most likely to be the real perpetrator. The information of the package is available at https://cran.r-project.org/package=likeLTD.

In DNA profiling, the alleles which present in the sample are determined by using a graph, called electropherogram (epg). An epg represents the DNA composition of the sample after it is amplified (Jamieson, 2016). When an allele in specific loci is detected, there will be a peak within the graph; the heights of these peaks infer the amount of DNA within the sample. However, the peak heights are highly variable when dealing with low-quality DNA, thus making them protocol-sensitive (Balding, 2013). In likeLTD, these problems are overcome by assessing whether an allele is present, uncertain, or absent within a loci (Balding, 2013). By combining these information from each loci, the likelihood of every hypothesis can be determined without using the peak height information.

The hypothesis in likeLTD is similar with the one from Lab Retriever; however, it is also able to consider the contribution of profiled possible contributors (e.g. victim) and up to two unprofiled individuals (denoted as U1 and U2) within the calculation. As a result, the hypotheses become:

\[
H_p = Q + K1 + K2 + U1 (+U2)
\]
\[
H_d = X + K1 + K2 + U1 (+U2)
\]

where \(Q\) is the suspect, \(X\) is the alternative of \(Q\), \(K1\) and \(K2\) are the profiled possible contributors, while \(U1\) and \(U2\), if any, are the unprofiled individuals. \(H_p\) denotes the prosecution hypothesis (suspect is the real perpetrator), while \(H_d\) is the defence hypothesis (another person is the real perpetrator).

In likeLTD, there are five parameters that are used: \(D(1)\), \(\alpha_s\), \(c\), contribution of DNA in correlation with the reference individual (usually the suspect), and \(\gamma\). \(D(1)\) is the probability of one ‘dose’ of allele to drop; it corresponds to the dropout of single heterozygote allele of the reference individual. \(\alpha_s\) represents the significance value of locus \(s\); it is calculated based on the following equation:

\[
\alpha^\beta_s = \frac{D(1)}{1 - D(1)}
\]

with \(\beta\) is estimated to be -4.35 (Tvedebrink et al., 2009). Moreover, \(c\) refers to the drop-in probability, which might be caused by environment contamination. Lastly, \(\gamma\) shows the gamma distribution of every contributor with allelic dropout. LikeLTD will then estimate the
maximum LR based on the given parameters, with penalties on \( \alpha_s, c, \) and \( \gamma_i \).

The likelihood of every contributor is determined by the weight of evidence (WoE) and represented as \( \text{bans} \). The ban is the unit of WoE where \( x \) bans means \( \log_{10}(LR) = x \) (Good, 1979); higher ban value means higher likelihood of the individual to be the perpetrator. With number of repetition \( (n) \) equals to 5000, likeLTD requires a few minutes up to several days to calculate the LR, depending of the presence of \( U_1 \) and/or \( U_2 \) (the value of \( n \) was set to be 5000 as it had good precision for the simulated data) (Balding, 2012). However, as the software is keep being updated (the current v6.3.0 is developed in February 9, 2018), the time complexity is expected to become much faster in the future.

**DISCUSSION**

In this review, the author lists four computational software that assess allelic dropout event in DNA, particularly STR, profiling: MicroDrop, LabRetriever, Pedant, and likeLTD. All of them implement LR algorithm to calculate the allelic dropout probability and/or the LR ratio. However, each software has its own assumptions and parameters. In MicroDrop, all of the missing and incorrect genotype are considered to be caused by allelic dropout (second assumption of MicroDrop); in fact, it is not always the case. Sometimes, the incorrect genotype might be caused by contamination or human error. LabRetriever identifies this aspect and include allelic drop-in probability within their calculation, which later be improved in likeLTD by adding penalty on it. Pedant has different approach; instead of considering drop-in event, it estimates false error rate, which is assumed to happen at most once in every duplication (fifth assumption of Pedant).

In terms of function, MicroDrop and Pedant has different output with the other two. MicroDrop and Pedant estimate the allelic dropout probability of the data, while LabRetriever and likeLTD utilize the dropout probability to determine the likelihood of a suspect to be the real perpetrator. However, as mentioned before, there has been no study that comprehensively compares and/or correlates these four software. It might be caused by the fact that each software does not use its own algorithm. Instead, they improved or changed the previous algorithm to match their needs. For example, the error model of Wang (2004) assumes that allelic dropouts always precede false allele when both of them occur in an allele; this assumption is reversed in the Pedant model. As the change is not huge, each software might produce slightly different results.

However, one important aspect that must be considered in choosing the software is the type of population data. In MicroDrop, the model is built based on the Native American data. According to Wang et al. (2007), Native American population has lower heterozygosity and fewer distinct allele per locus than the other region. As a result, the MicroDrop model might produce non-optimal allelic dropout rate when the population data has distinct heterozygosity than Native American population. This also applies for LabRetriever, as the available races are set: Caucasian, African-American, or Hispanic. Pedant does not include races within their calculation; however, it still requires the expected heterozygosity value \( (H_e) \) as an input, which correlates with the population population itself. The one that does not include this parameter within the calculation is LikeLTD.

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