`likeLTD` v6.3: an illustrative analysis, explanation of the model, results of validation tests and version history

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Abstract

`likeLTD` ("likelihoods for Low Template DNA profiles") is an R package for computing likelihoods for DNA profiles. Version 6.0 included both a discrete model, almost unchanged from Version 5.5, that uses allelic calls (present/uncertain/absent) inferred from an electropherogram by an expert, and a new continuous model that uses the peak heights directly. Both models can handle multiple profiled possible contributors and up to two unprofiled contributors, in addition to the queried contributor, as well as sporadic dropin. The continuous model explicitly accommodates stutter, double-stutter and over-stutter, which are typically called as uncertain or non-allelic when using the discrete model.

This document describes the continuous model of `likeLTD`, including the modelling of peak heights, accounting for the effects of stutter and DNA degradation, as well as installation and running of the software. Installation and parameter options are illustrated using example input files (the "Example case" and the "Laboratory case") that are provided with the package and are described below. Much of the information in this guide has been published (Steele and Balding, 2016), which additionally includes a test of the linkage adjustment we propose, investigation of the behaviour of the continuous model in relation to multiple replicates, and comparison of results with theoretical predictions of the model. For corresponding information about the discrete model see the guide for Version 5.5 and Balding (2013). For background on forensic DNA profiling see Butler (2010), and for introductions to statistical methods for evaluating DNA profile evidence see Buckleton et al. (2016); Balding and Steele (2015).

We present some comparisons of results from running both continuous and discrete models on a range of single-contributor and mixed laboratory-generated DNA profiles. We also present results from the continuous model on a subset of those profiles subject to modifications, such as alteration of heights of individual peaks, or inclusion of extra peaks. All results reported here, unless otherwise stated, are from running Version 6.1 of `likeLTD`, with a standard allele frequency database of around 7000 UK Caucasians, $F_{ST} = 0.03$, a sampling adjustment `adj` $= 1$, and a detection threshold of 20 RFU for all loci.

Changes in v6.3

Version 6.3 makes further improvements to the allele and output reports to simplify presentation of the crime scene profile (CSP) and known genotypes into a single table. The evaluation report now flags any parameter estimate that is close to the pre-specified bounds on the search space (the bound may need to be relaxed to achieve a better result). The default maximum heterozygous DNA contribution of an individual has been changed from 5000 to the maximum observed peak size in the CSP. A parameter to specify the minimum dropin value has been added. We include two new database files, covering 4 US populations for the Globalfiler and Identifiler kits. There is a minor bug fix in reporting rare alleles in the reports.

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1 Example analysis

1.1 Installation

Installing `likeLTD` (only needs doing once on any computer) and loading it (once per `R` session) are both very simple.

```
install.packages("likeLTD")
require(likeLTD)
```

The `install.packages` command may generate a request for you to choose a site from which to download the package. Choose any site near you.

1.2 Specifying the hypotheses to be evaluated

We wish to evaluate the evidence for a profiled individual Q to be a contributor to a crime scene DNA profile. To do this `likeLTD` computes a likelihood ratio (LR) of the form:

$$\mathrm{LR} = \frac{\Pr(E|H_p)}{\Pr(E|H_d)} \tag{1}$$

where E is the DNA evidence: the crime scene profile (CSP) and reference profiles for Q and any uncontested contributors (denoted K for known). Here, H_p is a prosecution hypothesis that includes Q and K as contributors of DNA to the CSP, while H_d is a defence hypothesis that is often obtained from H_p by replacing Q with X, where X denotes an unknown alternative to Q. Below we will report weight of evidence (WoE) $= \log_{10}(LR)$ in units called bans.

The hypothesis pair for a `likeLTD` analysis is specified in part by a reference file consisting of one or more profiles (one per row) with exactly one profile tagged as "queried" and any others as "known" in the second column (titled "known/queried"). Each homozygous locus in a reference profile should be input as two alleles e.g. "16,16". To consider a different hypothesis pair that varies Q or adds/removes K, a corresponding reference file must be created.

Specification of the hypothesis pair is completed by setting a number (0, 1 or 2) of unknown contributors (U) and whether dropin alleles will be modelled (see below). Adding additional U to H_p and H_d beyond the minimum necessary to explain the observed epg peaks increases computational burden but has little effect on the result. Cowell et al. (2013) illustrate this with an example in which a WoE of 14.09 bans with three contributors barely changes as the number of contributors increases, reaching 14.04 bans with eight contributors.

1.3 The Example case: data input

The first example that we use to illustrate `likeLTD` was new in v6.2 and is modified from a real-world crime case. We will refer to it as the "Example case". The data files can be found in directory `.../inst/extdata/example` where `...` is system specific (see `datapath` below).

Reference profiles at the 16 autosomal loci of the NGM SElect$^{\mathrm{TM}}$ PCR amplification kit are provided in file `exampleRef.csv` for one queried contributor (Q) and one uncontested contributor (K). The CSP, in input file `exampleCSP.csv`, consists of three replicate profiling runs at these loci. The contents of this file include columns for the designations of the alleles corresponding to observed electropherogram (epg) peaks, and for the heights of those peaks. There are also columns headed "size" that are ignored by `likeLTD`; these columns are created automatically by some epg analysis software.

```
datapath = file.path(system.file("extdata", package="likeLTD"),"example")
```

This command finds out where your system has stored the Example case files, and saves that location in `datapath`. For your own analyses, you will need to create your own CSP and reference files, in the same format as `exampleCSP.csv` and `exampleRef.csv`. It is usually most convenient to create these files in a specific directory, and then set that to be the working directory for `R` using the command `setwd()` or using the `R` menu option (its location varies across operating systems). For example if your case files are in the directory `C:/Users/JoeBloggs/Cases/JoeBloggs1` then enter the command `setwd("C:/Users/JoeBloggs/Cases/JoeBloggs1")`. After that you can set `datapath = "."` in place of the command above.

Next we specify parameters required for `likeLTD` to compute the allele report. The possible arguments for command `pack.admin.input.peaks` are

`peaksFile`: Path to CSP file with peak heights. No default.

`refFile`: Path to file with reference profiles. No default.

`caseName`: Case name. Defaults to "dummy".

`databaseFile`: Path to database file. Defaults to `NULL`.

`kit`: Only used if `databaseFile=NULL`. Choice of database supplied with `likeLTD` (see Section 1.8 for further details). Can take the values "DNA17" (default), "SGMplus", "Identifiler", "NISTidentifiler", "NISTglobalfiler". Set to `NULL` if `databaseFile` is not `NULL`.

`linkageFile`: Path to file containing recombination rates between linked loci. Defaults to `NULL`, which implies that the linkage file supplied with `likeLTD` will be used, which has been developed for use with the 3 UK database files. Only used when Q and X are related. See Section 1.9 for further details.

`detectionThresh`: Detection threshold used for analysing peaks. This is either a single value that is applied at all loci, or a named list giving the detection threshold at each locus. Defaults to a single value of 20 RFU.

`outputPath`: Output path for reports. Defaults to the current working directory.

For the Example case, we could use:

```
# File paths and case name for allele report
admin = pack.admin.input.peaks(
            peaksFile = file.path(datapath, 'exampleCSP.csv'),
            refFile = file.path(datapath, 'exampleRef.csv'),
            caseName = "Example",
            detectionThresh = 20
             )
```

Here, neither `databaseFile` nor `kit` has been specified, so `likeLTD` will use the default DNA17 database. If you wish to specify a different `detectionThresh` for each lane of the CSP the admin specification should be similar to:

```
# File paths and case name for allele report
admin2 = pack.admin.input.peaks(
            peaksFile = file.path(datapath, 'exampleCSP.csv'),
            refFile = file.path(datapath, 'exampleRef.csv'),
            caseName = "Example",
            detectionThresh = list(D10S1248=20,vWA=20,D16S539=20,D2S1338=20, # blue
                                   D8S1179=30,D21S11=30,D18S51=30, # green
                                   D22S1045=40,D19S433=40,TH01=40,FGA=40, # black
                                   D2S441=50,D3S1358=50,D1S1656=50,D12S391=50,SE33=50) # red
            )
```

Any peak with height below the specified detection threshold is removed from the CSP, and is not displayed in the allele report; a warning is displayed to notify that this has occurred.

1.4 Allele report

Designation	S	DS and OS
Non-allelic	$x < 0.05$	$x < 0.05$
Uncertain	$0.05 \leq x < 0.15$	$0.05 \leq x < 0.1$
Allelic	$x \geq 0.15$	$x \geq 0.1$

Table 1: Criteria for designating alleles in stutter (S), double-stutter (DS) or over-stutter (OS) positions as either non-allelic, uncertain or allelic when estimating `nUnknowns` (not used for computing likelihoods). x indicates the ratio of the stutter position peak height to the parent peak height.

An allele report for the Example case (`Example-Allele-Report-1.doc`), generated by the command

```
allele.report.peaks(admin)
```

with `admin` as defined above, is provided in (Appendix A).

The allele report is a .doc created in the current working directory (set `outputPath` to specify a different directory). It summarises the input data, highlights rare alleles, and suggests values for key parameters (and hence suitable hypotheses to compare), in particular it suggests values for `nUnknowns`, the number of unknown contributors required to explain the observed CSPs under H_p, and whether or not to model dropin.

In order to estimate `nUnknowns`, peaks are called as non-allelic, uncertain or allelic according to the criteria given in Table 1. Note that if over- and double-stutter are not modelled, manual evaluation of `nUnknowns` may be required. The DNA contribution of any unknown contributors is estimated using k-means clustering on the heights of peaks that are called as allelic and not attributable to Q or K, where k is the minimum number of unknowns based on those peaks. If an individual's DNA contribution is estimated at $< 1/3$ that of Q, we recommend that the individual can be modelled as dropin, see Section 4.3.5 for a demonstration that modelling minor contributors (relative to Q/X) as dropin has little effect on the resulting WoE.

For the Example case, the allele report indicates that, without allowing for dropin, three unknown contributors are required under H_p to explain the observed peaks. However, it is possible to check in the allele report (Appendix A) that almost all the peaks not attributable to alleles or stutters of Q or K are low: one is at 150 RFU and several are between 50 and 100, most are below 50 RFU. Therefore some unknown contributors can be modelled as dropin. In fact we confirm by an analysis using `nUnknowns=1` (Appendix B) that the heterozygote peak heights for the most prominent unknown contributor are estimated to be about 25 RFU.

1.5 Arguments and optimisation

Based on the allele report we specify the required hypotheses by setting the following arguments:

`nUnknowns`: The number of unknown contributors under the prosecution hypothesis (either 0, 1 or 2). `likeLTD` automatically adds an additional unknown contributor (X) under the defence hypothesis, who replaces Q from the prosecution hypothesis. Defaults to 0.

`doDropin`: Whether to model dropin or not (logical: `TRUE` or `FALSE`). Defaults to `FALSE`.

`ethnic`: The ethnic category of the queried contributor. The default database comes with "NDU1" (Caucasian),"NDU2" (African + Afro-Caribbean), "NDU3" (South Asian), "NDU4" (East Asian), "NDU6"

(African) and "NDU7" (Afro Caribbean). If you use your own allele frequency database you will choose your own category labels (required even if there is only one category). Defaults to "NDU1".

`adj`: Sampling adjustment (scalar). Defaults to 1.

`fst`: F_{ST} adjustment (scalar) for distant relatedness (coancestry) of Q and X. Defaults to 0.03.

`relationship`: Assumed relationship between Q and X. Can take values between 0 and 7 (defaults to 0):

0 Unrelated.

1 Parent/offspring.

2 Siblings.

3 Uncle (or aunt)/nephew (or niece).

4 Half-uncle (or half-aunt)/half-nephew (or half-niece).

5 Cousins.

6 Grandparent/grandchild.

7 Half-siblings.

The direction of relationships does not alter the computation, so is unspecified e.g. if `relationship=1`, the relationship may be Q as parent and X as offspring, or Q as offspring and X as parent.

`combineRare`: Whether to combine rare alleles that have not been observed in the CSP or reference profiles (logical: `TRUE` or `FALSE`). Defaults to `TRUE`.

`rareThreshold`: Allele probability below which unobserved database alleles will be combined when `combineRare` is set to `TRUE`. Defaults to 1, meaning all unobserved database alleles will be combined.

`doDoubleStutter`: Whether to model double-stutter (stutter to two repeat units smaller than the parent peak) or not. Defaults to `TRUE`.

`doOverStutter`: Whether to model over-stutter (stutter to one repeat unit larger than the parent peak) or not. Defaults to `TRUE`.

The default values for `combineRare` and `rareThreshold` cause all alleles in the database that were not observed in the CSP or reference profiles to be combined into an allele labelled "-1", which is given the mean longest uninterrupted stutter (LUS, see Section 3.2) and allele length (BP) of the combined alleles, and the sum of their probabilities. During computation if a joint genotype allocation shares n "-1" alleles, these are assumed to be n distinct alleles e.g. peak heights of unobserved alleles do not stack. Fewer unobserved alleles are combined leading to a more accurate calculation if `rareThreshold` < 1, but the improvement in accuracy is usually small and setting this parameter to a low value or setting `combineRare=FALSE` can greatly increase runtime when `nUnknowns=2`.

For the Example case we require dropin to be modelled, but if we otherwise accept all default values then the argument specification would be:

```
# Enter arguments
args = list(
      doDropin = TRUE
)
```

If instead we wished to include an unknown contributor of DNA and use a South Asian database, the `args` list would be:

```
# Enter arguments
args2 = list(
    nUnknowns = 1,
    doDropin = TRUE,
    ethnic = "NDU3",
    )
```

When `nUnknowns=1`, the two hypotheses for the contributors of DNA to the Example case CSP are:

$$\begin{aligned} H_p: &\quad \text{Q + K + U + dropin} \\ H_d: &\quad \text{X + K + U + dropin} \end{aligned}$$

where Q, X, K and U are all assumed unrelated to each other. Setting `nUnknowns=0` corresponds to removing the U from each hypothesis. We will use shorthand notation such as 1U + dropin and 0U + dropin to refer to these two hypothesis pairs. In the Example case, the allele report suggests 1U + dropin to obtain an LR that is a good approximation to that with 2U + dropin, but in fact 0U + dropin also leads to an excellent approximation to the LR in this case.

1.6 Running the Example case

After setting the arguments, we run some further preparatory commands:

```
# Create hypotheses
hypP = do.call(prosecution.hypothesis.peaks, append(admin,args))
hypD = do.call(defence.hypothesis.peaks, append(admin,args))
```

The function `do.call` calls the function given in its first argument. `prosecution.hypothesis.peaks` and `defence.hypothesis.peaks` are both functions defined within `likeLTD` to generate the objects needed by H_p and H_d respectively. By keeping args the same in defining hypP and hypD the two hypotheses differ only by swapping Q with X, which we find to be the most useful comparison in practice, but other hypothesis pairs can be compared by changing args between the two commands.

```
# Get parameters for optimisation
paramsP = optimisation.params.peaks(hypP)
paramsD = optimisation.params.peaks(hypD)
```

The function `optimisation.params.peaks` sets the parameters needed for optimisation. These values can be altered if required but the default settings should be adequate for most analyses. One possible exception is the argument `maxDropin` of `optimisation.params.peaks` which has a default of 250. This is the maximum total contribution of dropin to peak heights summed over all alleles (in RFU). This will be adequate for the low levels of dropin that typically arise. However we have shown (Steele and Balding, 2016) that minor contributors of DNA not of interest to the court can adequately be modelled as dropin (see Section 4.3.5), which may necessitate an increase in `maxDropin`. If the estimated dropin is close to the maximum, a warning will be displayed in the output report suggesting to re-run with a larger `maxDropin`. Similarly, if the epg was generated on a more sensitive genetic analyzer e.g. the ABI 3500 instead of the ABI 3130, then `maxDropin` may need to be increased.

All `likeLTD` commands until here should be fast. Now we are ready to do the main computation, which can be time-consuming. The output is stored in `results`:

```
# Run optimisation
results = evaluate.peaks(paramsP, paramsD)
```

The `evaluate.peaks` function is defined within `likeLTD`, and is a wrapper function for the `DEoptim` function that performs optimisation. The `evaluate.peaks` function splits the convergence into a number of steps, with each subsequent step having more stringent convergence tolerance and an increased crossover rate (a parameter for `DEoptim`); the combination of these two behaviours means that the parameter space is searched extensively to start with, and gradually focuses to a more intensive local search towards the end. The program stops running new steps after convergence has been reached, which is defined as having a relative difference between the current step result and all of the last `nConverged` (defaults to four) steps results less than `tolerance` (defaults to 1e-6). Interim results after each step are available when the argument `interim` is set as `TRUE` (default), which writes the most recent results to `Interim.csv`, and saves the internal state of the `evaluate.peaks` function to `interim.RData`, in the current working directory. The file `interim.RData` can then be handed to `evaluate.from.interim.peaks` to restart a computation that has partially completed. The seed to be used for optimisation may be specified by handing the `seed.input` argument to `evaluate.peaks`; if this argument is not specified then `likeLTD` sets the seed to a numeric representation of the current date, time and process ID.

The object returned by `evaluate.peaks` is a list of seven elements: `Pros`, `Def`, `WoE`, `Lp`, `Ld`, `seed.used`, `seed.input` and `runtime`. `Pros` and `Def` correspond to the prosecution and defence results respectively, and have the same structure as the object returned by `DEoptim` (see `help(DEoptim)`). The final WoE (in bans) can be obtained through the command `results$WoE[length(results$WoE)]`. `Lp` and `Ld` give the prosecution and defence likelihoods at each step. `seed.used` gives the seed that was used by the optimisation, while `seed.input` is `NULL` if no seed was specified but gives the user defined seed otherwise, so should be the same as `seed.used`. `runtime` is a list of three elements: `elapsed`, `start` and `end`.

Run time depends on many factors but the most important is the value of `nUnknowns`. The fact that there are three replicate profiling runs in the Example case also extends run time relative to a single run. We suggest that you initially accept the default value of `nUnknowns=0`, and then `evaluate.peaks` may require 1 to 2 hours, depending on your system. Later you can verify that the WoE is the same with `nUnknowns=1`, but the compute time may then be 6 to 12 hours. Run time is highly variable over cases and depends on whether your system is able to take advantage of multi-core processing. See Section 4.3.4 for further information. Running `R` in BATCH (background) mode, if available on your system, is convenient for longer runs.

1.7 Output report

```
# Generate output report
output.report.peaks(hypP,hypD,results)
```

The results are given in the output file `Example-Evaluation-Report-1.doc` (the numbering of the filename increments automatically, or a custom filename may be specified with `file="fileName.doc"`) which again summarises the input data, similar to the allele report, but also states the hypotheses compared, the single-locus and overall LRs and the WoE.

An output file for the Example case analysis with `nUnknowns=1` is given in Appendix B. The reported WoE of 18 bans corresponds to extremely strong support for the prosecution hypothesis that Q is a contributor of DNA to the CSP. The estimated DNA contributions are 169-170 RFU for Q/X, 1088-1092 RFU for K and 21-25 RFU for U. These RFU values correspond to an average height for a heterozygote allele. Dropin is 184-185 RFU, in this case it is the total peak height attributed to dropin over all alleles.

1.8 Allele frequency databases

A number of allele frequency database files are provided with `likeLTD`, in `.../inst/extdata/example`. These include databases for

- the 16-locus NGM SElect^{TM} database in 6 UK populations (`DNA17-db.txt`),
- the 10-locus SGM kit in 3 UK populations (`SGMplus-db.txt`),

- the 15-locus Identifiler kit in 3 UK populations (`Identifiler-db.txt`) and in 3 US populations (`NISTidentifiler-db.txt`), and
- the 21-locus Globalfiler kit in 4 US populations (`NISTglobalfiler-db.txt`).

The US data originates from NIST (National Institute of Standards and Technology) and was kindly put into `likeLTD` format by Keith Inman. The database files include: locus (marker) name, allele name (-100 denotes all rare alleles), LUS and bp (see below) and the allele frequency in each population. For details of populations included in each database run e.g. `help("DNA17-db")` for the DNA17 database.

Observed alleles that do not have either a LUS or BP value specified in the database will have these values extrapolated by `likeLTD`. LUS values will be extrapolated from the allele closest in size that shares the same partial repeat (allele 15 will be extrapolated from allele 14, allele 15.1 will be extrapolated from allele 16.1), if no allele shares the same partial repeat as the allele then the LUS value will be extrapolated from the allele closest in size.

These databases are provided for testing and illustrative calculations. To use your own database file instead (must be in same format) set `databaseFile` to the filename, including path if not in the working directory.

1.9 Linkage

Loci are said to be linked if they are located close enough on the same chromosome that allele transmissions from parent to child are correlated. If unaccounted for this tends to lead to an overstatement of the evidence against a Q who is closely related to an alternative contributor X. If the CSP contains linked loci, and Q and X are closely related (e.g. `args$relationship=2`) `likeLTD` will apply a correction factor to the LR of m_l/m_u where m_u is the match probability computed ignoring linkage and m_l is the match probability computed allowing for linkage. See Bright et al. (2013a) for details of the latter calculation.

This linkage correction will be close to correct for single contributor CSPs, or where the profile of Q forms a clear major component. In more challenging cases the `likeLTD` linkage correction will tend to over-correct, thus understating the evidence (Steele and Balding, 2016).

The linkage correction can be turned off by specifying `doLinkage=FALSE` in `optimisation.params.peaks`.

2 Genotype probabilities

```
# Get the most likely single-contributor genotypes
gens = get.likely.genotypes.peaks(hypD,paramsD,results$Def)
```

LikeLTD can provide a list of the most probable genotypes at each locus for each unprofiled contributor, using function `get.likely.genotypes.peaks`. By default only single-locus genotypes with probability > 0.1 are returned; this can be altered using the argument `prob`. The most probable whole-profile genotype, and its probability are also returned (Figure 1). The genotype probabilities are obtained as a by-product of computing the likelihood; genotype probabilities add no information to the assessment of WoE for an alleged contributor of DNA, but can be useful for searches in a database.

The returned list object is organised into a series of levels, as shown in Figure 1. It may also be desirable to obtain the joint probabilities of the genotypes of all unknown contributors, rather than marginal probabilities of the genotypes of individual unknown contributors. In this case, the argument `joint=TRUE` can be set in `get.likely.genotypes.peaks`. For joint probabilities, the threshold for genotypes at a locus to be reported is 0.05, but as for marginal probabilities, this can be altered by setting `prob`.

Some of the locus-specific genotypes used to construct the whole profile genotype may have probabilities below the threshold, and will therefore not be displayed in the locus-specific results.

```
# Return joint genotype probabilities and probabilities
gens = get.likely.genotypes.peaks(hypD,paramsD,results$Def,joint=TRUE)
```

```
[[1]]
[[1]][[1]]
[[1]][[1]]$D2S1338
[[1]][[1]]$D2S1338$genotypes
     [,1] [,2]
[1,] "17" "20"

[[1]][[1]]$D2S1338$probabilities
[1] 0.9466687


[[1]][[1]]$D21S11
[[1]][[1]]$D21S11$genotypes
     [,1] [,2]
[1,] "29" "32.2"

[[1]][[1]]$D21S11$probabilities
[1] 0.8802203


[[1]][[1]]$TH01
[[1]][[1]]$TH01$genotypes
     [,1] [,2]
[1,] "6"  "9.3"
[2,] "8"  "9.3"
[3,] "6"  "8"

[[1]][[1]]$TH01$probabilities
[1] 0.3114256 0.3108089 0.2920321



[[1]][[2]]
[[1]][[2]]$D2S1338
[[1]][[2]]$D2S1338$genotypes
     [,1] [,2]
[1,] "17" "24"
[2,] "20" "24"

[[1]][[2]]$D2S1338$probabilities
[1] 0.3723985 0.2639819


[[1]][[2]]$D21S11
[[1]][[2]]$D21S11$genotypes
     [,1] [,2]
[1,] "29" "31"
[2,] "31" "32.2"

[[1]][[2]]$D21S11$probabilities
[1] 0.5310860 0.2497111


[[1]][[2]]$TH01
[[1]][[2]]$TH01$genotypes
     [,1]  [,2]
[1,] "6"   "9.3"
[2,] "8"   "9.3"
[3,] "6"   "8"
[4,] "9.3" "9.3"

[[1]][[2]]$TH01$probabilities
[1] 0.2780060 0.2235599 0.2021422 0.1315162



$topGenotypes
$topGenotypes$genotypes
$topGenotypes$genotypes[[1]]
        [,1] [,2]
D2S1338 "17" "20"
D21S11  "29" "32.2"
TH01    "6"  "9.3"

$topGenotypes$genotypes[[2]]
        [,1] [,2]
D2S1338 "17" "24"
D21S11  "29" "31"
TH01    "6"  "9.3"


$topGenotypes$probabilities
$topGenotypes$probabilities[[1]]
[1] 0.2595038

$topGenotypes$probabilities[[2]]
[1] 0.05498282
```

Figure 1: Marginal genotype probabilities reported by `get.likely.genotypes.peaks` for a two-contributor, three-locus CSP. The first section of the results shows the single locus genotypes for single contributors in two subsections, one for each contributor subject to dropout (designated with `[[1]][[1]]` for the first contributor, and `[[1]][[2]]` for the second contributor). Each of these subsections is then further divided into locus sections (designated `$locusName`), which are then each split into genotypes and probabilities for that locus and that contributor. The probabilities correspond to the rows of the genotype matrices. The second section (designated `$topGenotypes`) shows the most probable whole-profile genotype for each contributor, and is split into genotypes and probabilities subsections, which are both further subdivided into contributors subject to dropout (e.g. `$topGenotypes$probabilities[[2]]` indicates the probability of the most probable genotype for the second contributor, which is displayed in `$topGenotypes$genotypes[[2]]`).

```
$joint
$joint$D2S1338
$joint$D2S1338$genotypes
     [,1] [,2]    [,3] [,4]
[1,] "17" "24"    "17" "20"
[2,] "20" "24"    "17" "20"
[3,] "24" "25"    "17" "20"
[4,] "24" "rares" "17" "20"
[5,] "23" "24"    "17" "20"
[6,] "19" "24"    "17" "20"

$joint$D2S1338$probabilities
[1] 0.34934859 0.23370053 0.07404989 0.07080871 0.07063617 0.06118081


$joint$D21S11
$joint$D21S11$genotypes
     [,1] [,2]   [,3] [,4]
[1,] "29" "31"   "29" "32.2"
[2,] "31" "32.2" "29" "32.2"
[3,] "31" "32.2" "29" "29"
[4,] "30" "31"   "29" "32.2"

$joint$D21S11$probabilities
[1] 0.49947197 0.16154539 0.08816570 0.08787847
```

```
$joint$TH01
$joint$TH01$genotypes
     [,1]  [,2]  [,3] [,4]
[1,] "6"   "9.3" "8"  "9.3"
[2,] "8"   "9.3" "6"  "9.3"
[3,] "9.3" "9.3" "6"  "8"
[4,] "6"   "8"   "6"  "9.3"
[5,] "6"   "9.3" "6"  "8"
[6,] "6"   "6"   "8"  "9.3"

$joint$TH01$probabilities
[1] 0.15656755 0.15078208 0.13151619 0.11307157 0.10921223 0.09908661


$topGenotypes
$topGenotypes$genotype
        [,1] [,2]  [,3] [,4]
D2S1338 "17" "24"  "17" "20"
D21S11  "29" "31"  "29" "32.2"
TH01    "6"  "9.3" "8"  "9.3"

$topGenotypes$probability
[1] 0.02731945
```

Figure 2: An example output of `get.likely.genotypes.peaks` for a two-contributor CSP, obtaining joint genotype probabilities. The first section (`$joint`) displays the joint genotype probabilities at each locus for those genotypes with probability $>$ `prob` (`$joint$locusName$genotypes`), as well as their associated probabilities (`$joint$locusName$probabilities`). Once again the probabilities correspond to rows of the genotype matrices. The second section (`$topGenotypes`) displays the most likely whole-profile joint genotype (`$topGenotypes$genotype`) as well as its associated probability (`$topGenotypes$probability`).

```
# Return joint genotype probabilities with per-locus probability greater than 3%
gens = get.likely.genotypes.peaks(hypD,paramsD,results$Def,joint=TRUE,prob=0.03)
```

The returned object `gens` is organised similarly to the single-contributor object, with the dependence on contributor removed from the organising hierarchy, as shown in Figure 2.

If there are three unprofiled contributors to the CSP, the function will return either a genotype list for each contributor if `joint=FALSE`, or a genotype list with six columns (two alleles for each contributor) if `joint=TRUE`. If there is only a single contributor to the CSP, the results will be the same regardless of the value of `joint`, although they will be displayed slightly differently.

3 The `likeLTD` peak height model

3.1 Overview

3.1.1 Key features of `likeLTD`

- `likeLTD` uses peak height information directly; providing similar or greater statistical efficiency than the discrete model (which remains available and was the only model prior to v6.0). There is a substantial improvement in statistical efficiency relative to the discrete model for some CSPs.
- It combines information across replicate profiling runs, thus avoiding the need for a "consensus" profile (Gill et al., 2000).
- DNA dose decreases with fragment length due to degradation, based on the model of Tvedebrink et al. (2012).
- Stutter ratio has a linear relationship with longest uninterrupted sequence (LUS), as demonstrated by (Kelly et al., 2014), and this relationship is allowed to differ both across loci and across replicates.

- As a consequence of estimating the DNA contribution, a potential contributor can be considered in a hypothesis without implying that their DNA is present, because the contribution of DNA from that individual can be estimated at zero.

- Because the penalised likelihoods are maximised over the nuisance parameters, combining information over alleles, loci, replicates and individuals, there is little need for external calibration data. This is only required for a few hyperparameters – the parameters of the penalty functions. The underlying parameters are allowed flexibility to best fit the CSP data under each hypothesis, constrained by penalty functions that depend on these hyperparameters.

3.1.2 The contributors of DNA

Given the CSP and reference profiles, we seek to compare the likelihood when a profiled individual Q is a contributor to the CSP with the corresponding likelihood when Q is replaced by an unprofiled individual X. The ratio of those two likelihoods, each maximised over the nuisance parameters, is the likelihood ratio (LR). There can be up to two further unprofiled possible contributors of DNA, U1 and U2, and multiple profiled uncontested contributors (K1, K2, ...).

There can be several LRs of interest, considering X of different ethnicities and different relatedness with Q (the more genetically similar X is from Q, the smaller the LR). `likeLTD` allows X to be related to Q with the specification of one of eight possible relationships. In addition, we use an F_{ST} adjustment to allele fractions that allows for possible remote shared ancestry of Q with X. Within `likeLTD`, this adjustment only affects the alleles of Q and does not take into account any other profiled contributors. We assume U1 and U2 to be mutually unrelated, and they and the K are all assumed unrelated to X (when these individuals are included in both hypotheses, any relatedness to X will usually have little effect on the LR).

Because the relatedness coefficients and F_{ST} account for the positive correlations across loci due to shared ancestry of Q and X, it is reasonable to compute full-profile LRs by multiplication of single-locus LRs, which is standard practice in the assessment of DNA profile evidence (Buckleton et al., 2016). We thus focus below on the single-locus case.

3.1.3 The parameters

The "nuisance" parameters, which must be eliminated under each multi-locus likelihood before taking their ratio, are

- the DNA contributions of each hypothesised contributor in RFU.

- the parameters of the stutter model; mean gradient and multiplicative locus adjustment.

- the mean double- and over-stutter fraction, if modelled.

- one degradation parameter for each hypothesised contributor, and one degradation parameter for dropin peaks, if modelled.

- a multiplicative replicate adjustment; one for each replicate after the first, with the first as the "reference" replicate.

- a dropin dose (RFU), if modelled.

- the scale parameter for the gamma distribution, used to compute probabilities of observed peak heights given the expected peak height.

`likeLTD` maximises a (penalised) likelihood over these parameters using the `R DEoptim` function.

3.1.4 Dropin model

The dropin parameter in `likeLTD` is the expected total contribution of dropin to peak heights at a locus in one profiling run. Because dropin is ubiquitous for low-template profiles the default minimum dropin dose is 5 RFU and the default maximum is 250 RFU. Dropin of a given allele is assumed to occur in proportion to the population frequency of that allele. Thus, if the dropin value is λ, then the dropin contribution to the peak height at an allele with population fraction p is λp RFU. This dropin dose is subject to degradation at a different rate from the degradation rates of contributors, but the degradation model is the same: the effect of degradation is to multiply expected peak height by $(1+\delta)^{-f}$, where δ is the dropin-specific degradation parameter and f is the mean adjusted fragment length of the allele in base pairs.

3.2 Further details

Computations are performed separately under H_p and H_d. Let C denote the set of contributors under a given hypothesis. Suppose that the CSP replicates are indexed by the elements of a set R, and include loci in the set L, while I_l denotes the set of possible alleles at locus $l \in L$. Each element of G_l is an allocation of genotypes at locus l to each $c \in C$. The genotype of Q is constant over G_l, and similarly for other c with reference profile available, but the elements of G_l vary according to the genotypes allocated to unprofiled c. Population genotype probabilities are assumed given. In practice, allele probabilities are obtained from a database, possibly using a sampling adjustment, and genotype probabilities are derived as products of allele probabilities assuming Hardy Weinberg equilibrium, possibly with an F_{ST} adjustment (Balding and Steele, 2015).

Let χ_c denote the effective DNA mass at a heterozygote allele of $c \in C$ in the first replicate, expressed in RFU, a unit of peak height. To compute the expected contribution from c to the height of an epg peak at allele $i \in I_l$ for a given $g \in G_l$, we first adjust for the genotype of c specified by g, the replicate $r \in R$, and DNA degradation:

$$P_{l,r,g,c,i} = \frac{n_{g,c,i}\rho_r\chi_c}{(1+\delta_c)^{f_i}}, \tag{2}$$

where $n_{g,c,i} \in \{0,1,2\}$ indicates the number of i alleles in the genotype of c and ρ_r denotes a replicate adjustment ($\rho_1 = 1$), while δ_c is a parameter measuring the degradation of DNA from c and f_i is the mean adjusted length of allele i in base pairs. Each $P_{l,r,g,c,i}$ must next be adjusted for the fractions that stutter to allelic position $i-1$ (S), double-stutter to $i-2$ (D) or over-stutter to $i+1$ (O). Whereas D and O are global constants, because these are rare events and it would be difficult to parametrise the relationship, we propose a zero-intercept linear model for S:

$$S_{l,i} = \alpha_l u_i.$$

Here, α_l is the locus-specific coefficient of u_i, the longest uninterrupted sequence (LUS) of allele i (Brookes et al., 2012; Bright et al., 2013b; Kelly et al., 2014). To compute the expected peak height at allele i in replicate r for a given g, each $P_{l,r,g,c,i}$ is incremented with any stutter contribution from allele $i+1$, double stutter from $i+2$ and over-stutter from $i-1$, and summed over contributors c. Finally, a contribution from dropin is added. This gives the expected peak height as:

$$E_{l,r,g,i} = \frac{\lambda p_i}{(1+\delta)^{f_i}} + \sum_{c\in C}((1 - S_{l,i} - D - O)P_{l,r,g,c,i} + S_{l,i+1}P_{l,r,g,c,i+1} + DP_{l,r,g,c,i+2} + OP_{l,r,g,c,i-1}). \tag{3}$$

where p_i is the population allele fraction and λ is a dropin parameter, in RFU. Note that dropin of an allele is assumed to occur in proportion to its population frequency, and is adjusted for degradation with a dropin-specific rate δ.

The peak height at allelic position i is then assumed to have a gamma distribution with expectation $E_{l,r,g,i}$ and variance $\sigma E_{l,r,g,i}$. The scale parameter σ is a global constant, so that values of l, r, g and i affect

peak-height variance only through the mean. In `likeLTD` we treat peak heights as discrete: observed values are recorded to the nearest integer RFU value, say j, and we compute the corresponding probability as the gamma probability mass between $j-0.5$ and $j+0.5$. The dropout probability is the gamma probability mass assigned to the interval $(0, t_l-0.5)$, where t_l is the detection threshold (the smallest recordable peak height).

In `likeLTD`, alleles that are not observed in any CSP replicate or any reference profile of an assumed contributor are combined into a single allelic class. When the unprofiled contributors are assigned > 1 allele in this class, these are assumed to be distinct: unprofiled contributors are assumed not to share any unobserved allele.

Parameter	Distribution	Mean	SD
$\mathrm{E}[\alpha_l]$	N	0.013	0.010
$\log_{10}(\alpha_l/\mathrm{E}[\alpha_l])$	N	0	0.300
D	Γ	0.02	0.019
O	Γ	0.02	0.019
δ	e	0.02	0.020
σ	e	100	0.010

Table 2: Penalties applied to the parameters of the peak-height model. Distributions: N=normal, Γ=gamma, e=exponential. The degradation parameters δ have the same penalty for each contributor and for dropin.

In order to encourage the optimisation algorithm to search in realistic regions of the parameter space, the penalty terms shown in Table 2 are imposed. Large values of δ and σ are penalised, while for both D and O a zero value is excluded but a broad range of positive values is supported. Two separate penalties on the α_l are intended to allow flexibility for its mean while limiting its variance over loci. Incorporation of these penalty terms into the likelihood function is analogous to imposing a prior distribution, but our approach is not Bayesian: elimination of nuisance parameters is achieved via maximisation and not integration, which is for example the approach adopted by `STRmix`, implemented using Markov chain Monte Carlo.

The probability assigned to allelic position i, whether or not there is an observed above-threshold peak, is computed as a gamma probability mass as described above. Denoting this probability $a(l, r, g, i, \sigma)$, the penalised likelihood is computed by multiplying over alleles and replicates, summing over genotype allocations each multiplied by the product of genotype probabilities for the unprofiled contributors, and then multiplying over loci including the penalty term:

$$\prod_{l\in L} \pi_l \sum_{g\in G_l} \Big[\prod_{c\in C} Pr(\mathcal{G}_{g,c}) \Big] \prod_{r\in R} \prod_{i\in I_l} a(l, r, g, i, \sigma) \tag{4}$$

where $\mathcal{G}_{g,c}$ denotes the genotype allocated to c in g, while π_l is the combined penalty on the likelihood at locus l given the values for α_l, D, O, σ and the δ. (4) is then maximised over these parameters. `likeLTD` uses a genetic algorithm `Deoptim` that simulates mutation, recombination and selection on parameter vectors to search for the vector that maximises the penalised likelihood (Mullen et al., 2011). Maximisation is performed separately under H_p and H_d and the LR is the ratio of the maximised values.

4 Validation

To validate the peak height model we have carefully designed a series of tests to verify that the model adheres to expected behaviours under a number of conditions. We use the "Laboratory case", which is a laboratory-created mixture of three individuals who contribute approximately 250, 62 and 16pg of DNA. The input files are supplied with `likeLTD` in `.../inst/extdata/laboratory`. Reference profiles are available in input file `laboratory-reference.csv` for the 250pg contributor, who we treat as a known individual K, and the 16pg contributor who we treat as Q. The 62pg contributor is treated as U for all analyses (no reference

profile is provided). The CSP consists of a single profiling run of the NGM SElectTM PCR amplification kit and is available in input file `laboratory-CSP.csv`.

We first verified that the optimised model adheres to expected behaviour (Section 4.1). We then altered the model assumptions used to run the case and ensured that any resulting change in the WoE is consistent with the altered model assumptions (see Section 4.2).

Next, we generated a large number of laboratory CSPs ranging from one to three contributors, and have compared the WoE under both peak height and discrete models for each contributor in each CSP (Section 4.3), and verified that a greater WoE is obtained using the peak height model for unequal-contribution CSPs and similar WoE for equal-contribution CSPs. Subsequently, we altered a single peak at a time in one of the laboratory-generated CSPs and evaluated the WoE using the peak height model (Section 4.4). We verified that introducing peaks consistent with H_p increases the WoE, while removing peaks consistent with H_p and introducing peaks inconsistent with H_p both decrease the WoE.

4.1 Model fit

For the Laboratory case, `likeLTD` returns a WoE of 8.2 bans (Table 3, column 1) indicating extremely strong support for H_p, despite the low DNA contribution of approximately 16pg for Q and the complex nature of the CSP. The strongest support for H_p is seen at D21 and D12; both loci where the alleles of Q are not masked by allelic peaks of the major contributors. Conversely, D22 and D19 support H_d; at D22 Q is homozygous and masked by a major allele, so `likeLTD` explains the over-stutter at 17 as allelic for X under H_d (with a correspondingly lower $\widehat{\theta}$ under H_d), while at D19 Q has dropped out an allele while the corresponding 15 allele is observed unmasked which `likeLTD` finds to be more likely explained as X being heterozygous for 15 and a non-15 allele that is masked by one of the major contributors.

We assess the fit under H_d of the optimised `likeLTD` model for this case by observing the fraction of observed peak heights that lie within the central 50% and 95% intervals of their fitted gamma distributions, given both the most likely joint genotype allocation and the fitted parameters. The fit of the optimised parameters to the observed data can be investigated using the `peaks.results.plot` function included with `likeLTD`. This function plots boxplots for each hypothesised peak assuming the most likely joint genotype allocation, with boxes displaying the central 50% (inter-quartile range) of the gamma distribution, whiskers displaying the 95% equal-tailed probability interval, and red bars indicating the observed peak heights.

For the Laboratory case under H_d the proportions of observed peaks within the 50% and 95% probability intervals were 0.51 and 0.94, both close to their respective expected value (Figure 3).

4.2 Altering the model

Here we alter the assumptions of the model used to evaluate the WoE in the Laboratory case, modelling all combinations of double- and over-stutter with dropin, and removing the locus dependency of the stutter gradient. We expect that removing modelling assumptions that have no explanatory power for the given CSP to return an unaltered WoE, while removing modelling assumptions that are necessary to fully explain the CSP will results in an altered WoE.

Modelling dropin does not change the WoE for the Laboratory case (Table 3), as dropin is not necessary to explain the CSP when double- and over-stutter are both modelled, as evidenced by the dropin estimates of 5 and 5 RFU under H_p and H_d respectively, equal to the minimum dropin value of 5.0. Similarly, removing double-stutter from the model does not change the WoE as there are no peaks in the CSP that can only be explained through double-stutter. Conversely, removing over-stutter from the model reduces the WoE, particularly because the 17 peak at D22 can no longer be explained by over-stutter (D22 WoE decreases from -0.5 bans with SDO to -0.8 and -0.7 bans with SD and S respectively), so must be assumed to be allelic by the program. D22 is subject to over-stutter more commonly than any other locus in the NGM Select$^{\text{TM}}$ kit due to being the only locus with repeat units that are three base pairs long, rather than the standard four base pairs. In the peak height model the stutter ratio is assumed linear with the longest uninterrupted sequence (LUS) of the allele, with the gradient of the linear relationship allowed to differ between loci. When the stutter gradient is instead assumed to not vary between loci ($\alpha_l = E[\alpha_l]$) the WoE

Model	SDO	SDO+dropin	SO+dropin	SD+dropin	S+dropin	$\alpha_l = E[\alpha_l]$
Parameters						
Dropin	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE
DS	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE
OS	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE
WoE						
D10S1248	0.6	0.6	0.6	0.6	0.6	0.6
vWA	1.0	1.0	1.1	1.1	1.1	1.1
D16S539	0.5	0.5	0.5	0.5	0.5	0.5
D2S1338	0.5	0.5	0.5	0.4	0.4	0.6
D8S1179	1.1	1.1	1.1	0.9	0.9	1.1
D21S11	1.7	1.7	1.7	1.7	1.7	1.7
D18S51	1.0	1.0	1.0	1.0	1.0	1.1
D22S1045	-0.5	-0.5	-0.5	-0.8	-0.7	-0.5
D19S433	-2.8	-2.8	-2.8	-2.8	-2.8	-2.8
TH01	0.5	0.5	0.5	0.5	0.5	0.5
FGA	0.6	0.6	0.6	0.5	0.5	0.5
D2S441	1.2	1.2	1.2	1.2	1.2	1.2
D3S1358	0.7	0.7	0.7	0.7	0.7	0.7
D1S1656	0.7	0.7	0.7	0.7	0.7	0.7
D12S391	1.4	1.3	1.4	1.3	1.3	1.3
SE33	0.1	0.1	0.1	0.1	0.1	0.1
Overall	8.2	8.2	8.2	7.6	7.7	8.4

Table 3: Locus and overall WoE for the Laboratory case provided with `likeLTD`, under different modelling assumptions. Columns four to six alter whether double or over stutter are being modelled while in column seven the stutter gradient is constant over loci (see Section 4.2).

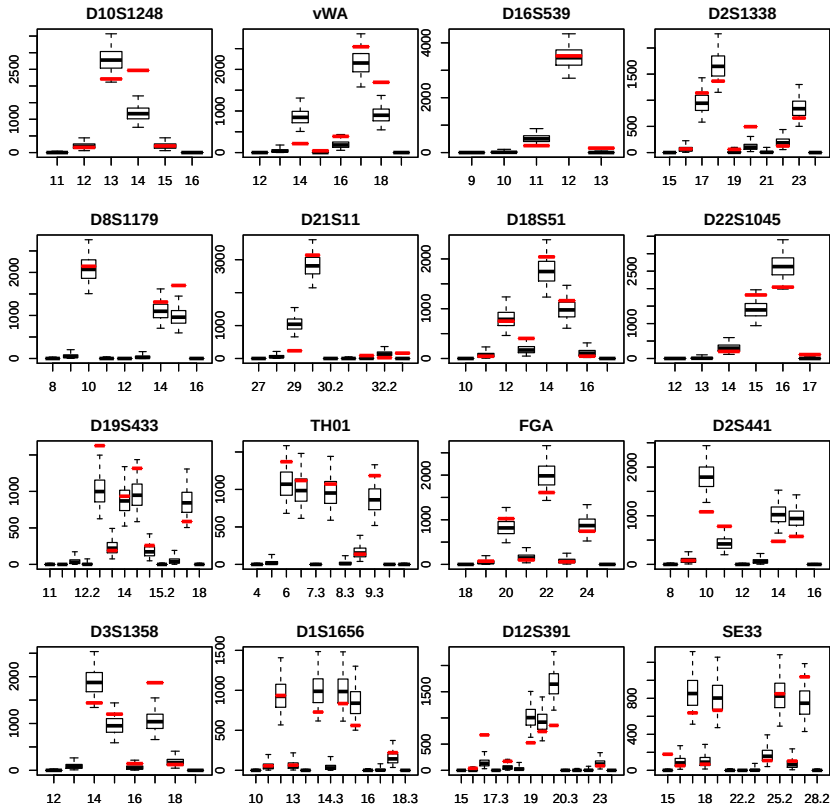


Figure 3: Boxes show the central 50% (inter-quartile range) of the gamma distribution for each hypothesised peak, whiskers represent the 95% equal-tailed probability interval and red bars show observed peak heights. RFU is displayed on the y-axes while allele labels corresponding to boxplots are displayed on the x-axes.

increases to 8.4 bans. This change in WoE is driven by the defence likelihood at D2S1338; at this locus Q is 17,22 but the most likely genotype for X is 17,18 meaning that the truly allelic peak at 22 is estimated to be stutter from one of the majors under H_d (K1=18,23), requiring a large stutter gradient which is not possible when the stutter gradient cannot vary by locus. This means that the defence hypothesis has a higher likelihood at D2S1338 when the stutter gradient is allowed to vary by locus, leading to a lower locus LR with a locus variant gradient (0.46) than with a fixed gradient (0.61).

We have demonstrated here that modelling dropin and removing the modelling of double-stutter does not change the WoE for the Laboratory case, as these phenomena are not required to explain this particular CSP. Conversely removing the modelling of over-stutter or locus-dependent stutter gradients has an effect on the WoE as these phenomena are important in explaining the CSP under either H_p or H_d. This fits the expected behaviour of explanatory modelling assumptions altering the WoE and non-explanatory modelling assumptions having no effect on the WoE.

4.3 Laboratory validation

Here we compare the results of the peak height and discrete models on a set of 72 one to three contributor CSPs that were laboratory generated. We expect the two models to provide similar results for many cases, but the peak height model is expected to return a higher WoE in favour of a true hypothesis when the peak heights are informative, such as when Q contributes much less DNA to the CSP than one or more other contributors.

Single-, two- and three-contributor CSPs were generated in the laboratory (see Appendix C) from the DNA of 36 donors. Single-contributor CSPs were created at DNA contributions of 4, 16, 62 and 250pg, with nine CSPs at each level. Two-contributor CSPs were created at both 16:250pg (12 CSPs) and 31:31pg (12 CSPs) DNA contribution ratios. Three-contributor CSPs were created at both 16:62:250pg (six CSPs) and 31:31:31pg (six CSPs) DNA contribution ratios. The WoE for each resulting CSP was evaluated using both discrete and continuous models of `likeLTD`. For multi-contributor CSPs, each contributor was queried in turn, leading to 36, 48 and 36 evaluations for the single-, two- and three-contributor CSPs respectively.

Here, the WoE will be presented as an information gain ratio (IGR) which is WoE/$\log_{10}$IMP, where IMP is the inverse match probability, the theoretical maximum LR for a given Q. This allows for intuitive comparison of the WoE across different queried individuals.

4.3.1 Single contributor

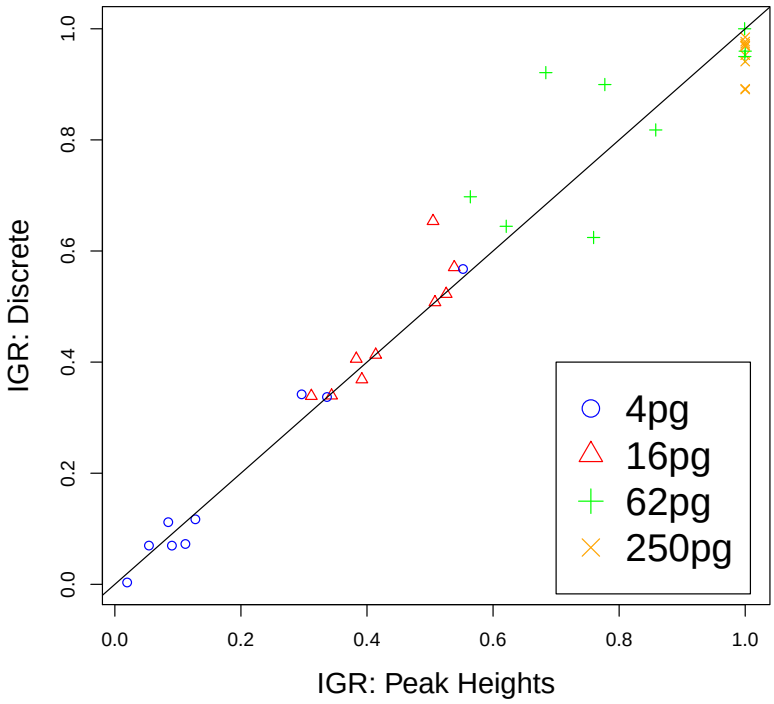


Figure 4: Information gain ratio (WoE/IMP) for 36 single-contributor CSPs using both the peak height (x-axis) and discrete (y-axis) models. Legend indicates the approximate DNA mass used to generate the CSPs.

IGR increases as the DNA mass increases, for both the peak height and discrete models (Figure 4). IGR is approximately equal between the two models for the majority of CSPs. At 16pg there is one exception to this equality, in which the discrete model returns a larger WoE than the peak height model, while greater variability is seen at 62pg. At 250pg the peak height model outperforms the discrete model for many CSPs because a minority of stutter peaks have been called as allelic, while many more have been called as uncertain. On reviewing the underlying CSPs, we found that in general the discrete model outperforms the peak height model when there is high variability in the observed CSP peak heights because the variance of the peak height model is constrained through a penalty on σ while the discrete model ignores peak height.

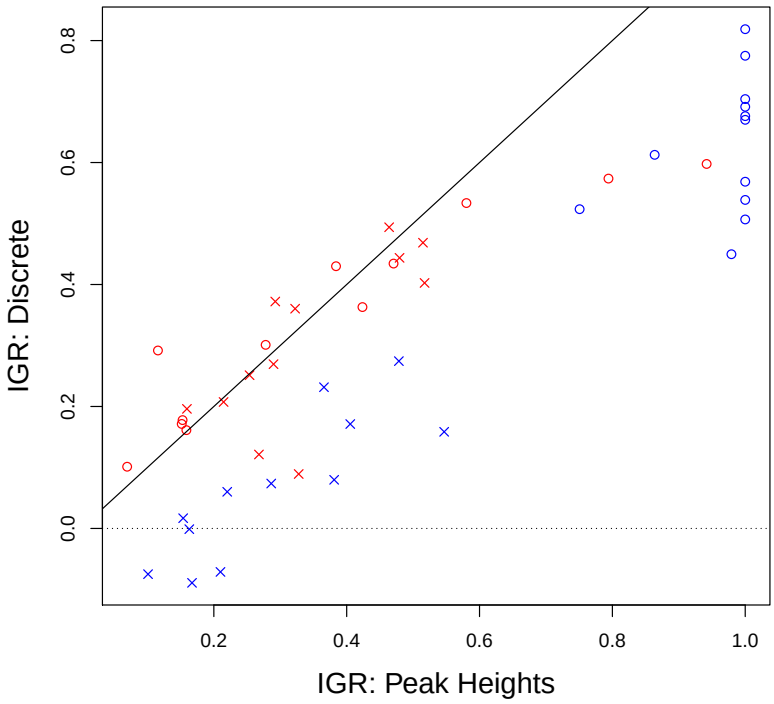


Figure 5: Information gain ratio (WoE/$\log_{10}$IMP) for 12 two-equal-contributor CSPs (red) and 12 two-contributor major/minor CSPs (blue) using both the peak height (x-axis) and discrete (y-axis) models. Both contributors to each CSP were queried, with circles and crosses indicating the first and second contributor respectively.

For instance, some of the CSPs included loci where Q was heterozygous, but a single large peak was observed, while the other allele had dropped out, which in reality requires a high variance but may instead be well explained as a homozygote under H_d. Contrastingly, the peak height model outperforms the discrete model when an allele has been misassigned as allelic for the discrete CSP.

4.3.2 Two contributors

The IGR is approximately equal using the peak height and discrete models when the equal-contribution CSPs are queried (Figure 5, red). Two of the equal-contribution cases in Figure 5 localise with the major/minor cases. Visual inspection of the CSPs indicated that there was in fact a large discrepancy in contributions despite the intention to create equal contributions, perhaps due to pipetting error. One CSP performs noticeably better with the discrete model than with the peak height model; once again visual inspection revealed an unusually high variation in peak heights causing the peak height model to be conservative because very high variability is penalised in the model.

All of the major/minor CSPs return an IGR that is larger with the peak height model than with the discrete model (Figure 5, blue). Two of the major-queried evaluations have an IGR < 0.9; each of these CSPs have been confirmed by manual inspection to have peak heights closer to equal contributions than suggested by the specified DNA contributions of 16pg and 250pg. Note that when the minor is queried, four CSPs support H_d (IGR$<$0) using the discrete model, but support H_p using the peak height model; we know that H_p is true in all of these cases. Similarly, when querying the major contributor, the discrete model IGR

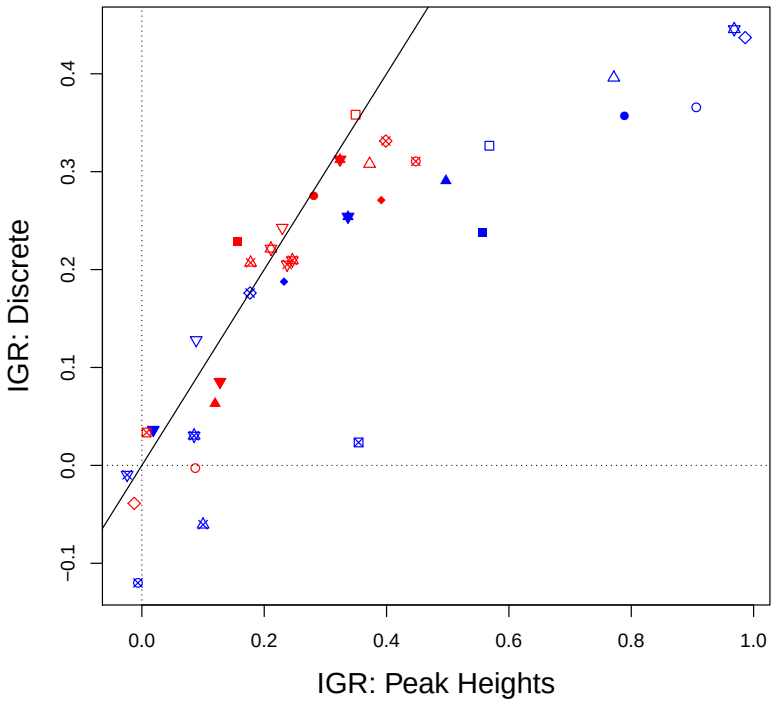


Figure 6: Information gain ratio (WoE/IMP) for 6 three-contributor equal-contribution CSPs (red) and 6 three-unequal-contributor CSPs (blue) using both the peak height (x-axis) and discrete (y-axis) models. The six cases of each condition are represented by square, circle, up-triangle, down-triangle, diamond and star symbols. Empty, filled and crossed symbols indicate that the first, second and third contributor were queried.

ranges from 0.4 to 0.8, while the peak height model is able to obtain close to full information (IGR=1.0) for the majority of CSPs, reflecting the fact that the peak height model is able to exploit more information in the CSP than the discrete model.

4.3.3 Three contributors

Of the six unequal-contribution CSPs evaluated (Figure 6, blue) one was a CSP for which whole-locus dropout was observed at 13 of the 16 used loci (downwards triangle), for which the peak height model is slightly more conservative than the discrete model for all evaluations perhaps due to insufficient information in the few observed peaks to estimate parameters of the model. Ignoring these three evaluations, all five 250pg-queried evaluations return a greater IGR with the peak height model than with the discrete model, all five do so for the 62pg-queried evaluations, and four of the five do so for the 16pg-queried evaluations with approximate equality in the 5th case. One 16pg-queried evaluation supports H_p using the peak height model, but supports H_d using the discrete model while in two 16pg-evaluations both the peak height and discrete models support H_d, despite H_p being true.

When equal-contributions CSPs are queried (Figure 6, red), the peak height and discrete models return approximately equal IGRs for all evaluations. Although peak heights can potentially distinguish single from multiple copies of an allele among the contributors (e.g. heterozygote from homozygote), in practice these results indicate that the variability in peak heights means that there is in fact little usable

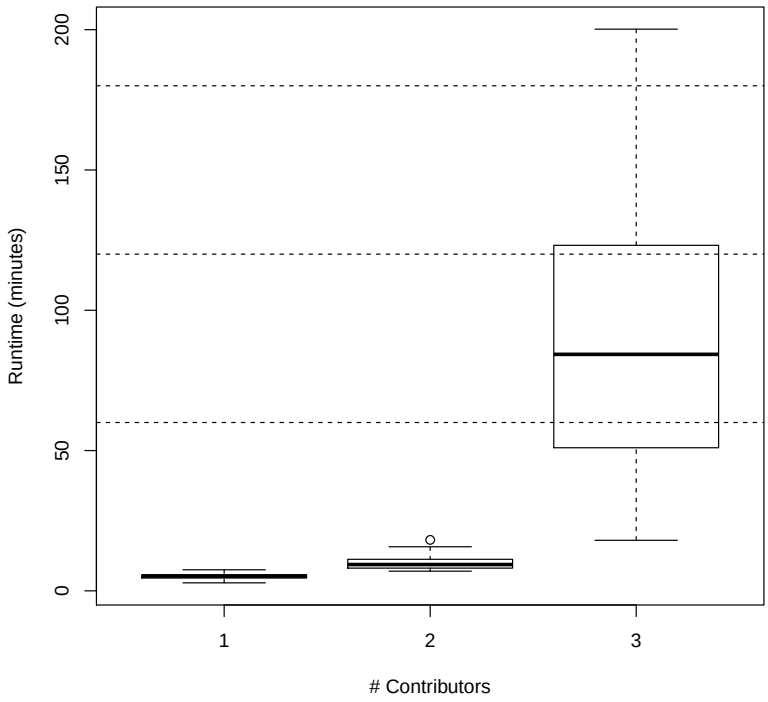


Figure 7: Runtime for the laboratory validation evaluations. Horizontal dashed lines indicate whole hours. The single- and two-contributor hypotheses included dropin, while the three-contributor hypotheses did not.

information in the equal-contributor scenario. There is one evaluation for which the peak height model supports H_p while the discrete model supports H_d, and one evaluation for which both the peak height and discrete models support H_d, despite H_p being true.

4.3.4 Runtime

The runtime for the peak height model over all laboratory validation evaluations ranges from 3 to 200 minutes, increasing with the number of contributors to the CSP (Figure 7). The runtimes for the peak height model are longer than for the discrete model (not shown here), but the peak height model can give substantially larger WoE for the true hypothesis under certain situations (see Section 4.3).

The run time for the peak height model scales with the number of observed peaks, the number of unknown contributors and the number of replicates in the CSP. Modelling over-stutter or double-stutter increases run time. The run times in Figure 7 were obtained on a desktop computer with 15Gb of RAM, and an eight core Intel i7 processor (at 3.1GHz per core). Computing times may vary across machines. See Sections 4.5 for further information regarding run times.

4.3.5 Minor as dropin

In some scenarios it may be possible to model peaks from a minor contributor as dropin rather than as an extra contributor. This not only reduces the computational complexity of the WoE calculation, but also eliminates the sometimes difficult decision of whether to treat low-level epg peaks as dropin or as an extra unknown contributor. The minor-as-dropin procedure is analogous to increasing the detection threshold to

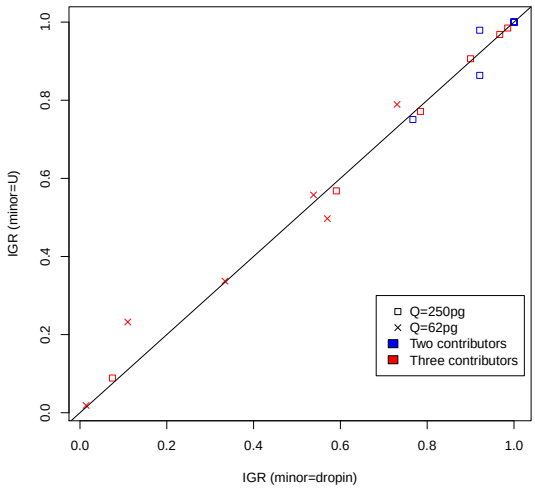


Figure 8: Information gain ratio (IGR) for 12 two- and/or 6 three-contributor CSPs (blue and red respectively) treating the minor contributor as dropin (x-axis) and as an additional contributor (y-axis).

eliminate low-level peaks that are not of interest, as is sometimes performed currently for complex epgs. Additionally, this procedure reduces the difficulty of assigning the number of contributors to a mixture, as any number of low-level contributors can be modelled as dropin. This also allows CSPs with more than two unknown contributors to be evaluated by `likeLTD`, provided that Q is not the minor contributor.

We re-evaluate the unequal-contributions two- and three-contributor CSPs with each contributor other than the minor as Q, demonstrating that low-level non-Q contributors can be explained as dropin with little-to-no effect on WoE against Q compared to treating them as unknown contributors (Figure 8).

4.3.6 Summary

The results presented here demonstrate that for a large number of laboratory CSPs the peak height model behaves as expected when compared to the discrete model; equal contribution mixtures return similar IGRs with both models, while the peak height model is able to utilise extra information in unequal contribution CSPs to return greater IGRs than the discrete model in favour of a true hypothesis. The peak height model (correctly) provides support for H_p in 16/18 minor contributor evaluations of laboratory-generated CSPs (16pg), and also does so in 41/42 equal contribution low-template evaluations (31pg), which is impressive given the very low DNA template levels. This is particularly useful for minor contributors, as highlighted by a number of cases where the discrete model supports H_d but the peak height model correctly supports H_p.

4.4 Validation using artificial changes to input data

Here we select one of the laboratory generated CSPs which we alter one peak at a time to verify that the resulting change in WoE is as expected. We expect that introducing dropped out alleles of Q will increase the WoE against Q, dropping out an allele of Q will decrease the WoE against him, introducing a dropin allele will decrease the WoE against Q, and that changes in peak heights that require greater variance of peak heights to explain the CSP under H_p should decrease the WoE and vice versa.

The single contributor CSP from donor 26 (16pg DNA) was used to investigate the behaviour of the peak height model when altering the CSP, as it had a mixture of locus dropouts (both heterozygote and homozygote), single dropouts (heterozygote) and non-dropouts (both heterozygote and homozygote). See Table 4 for a summary of the changes made to the CSP throughout this section.

Locus	G_Q	CSP	Observation	Alteration
D16	13,13	∅	Dropout of homozygous 13 allele	Reintroduction of 13 allele
				Introduction of 11 or 15 dropin peak
D18	14,17	14	Dropout of heterozygous 17 allele	Reintroduction of 17 allele
				Introduction of 8 or 12 dropin peak
D22	15,17	15,17	Fully observed heterozygote	Alteration of peak height at allele 17
				Introduction of 16 or 19 dropin peak
D19	13,14	∅	Full heterozygous dropout	Reintroduction 13 allele
				Introduction of 15 or 18 dropin peak
TH01	6,6	6	Observed homozygote allele	Alteration of peak height at 6
				Introduction of 8.3 or 9.3 dropin peak
FGA	23,25	25	Dropout of heterozygous 23 allele	Alteration of peak height at 25
				Introduction of 21 or 22.1 dropin peak

Table 4: Alterations applied to a single-contributor 16pg CSP at six loci. G_Q indicates the genotype of Q, the true contributor. ∅ under CSP indicates no observed peaks above the detection threshold at that locus. Observation gives the true effect seen at the locus. Alteration gives the two changes that were made at each locus. Reintroductions of dropped-out alleles ranged from 0 to 61 RFU, introductions of dropin peaks ranged from 0 to 61 RFU and alterations of observed peaks ranged from 0 to 151 RFU.

4.4.1 Missing peak insertion

A peak at the position of a single allele of Q which had dropped out was inserted into the CSP with varying peak height. This was done at three separate loci with:

1. No observed peaks, Q is homozygous (D16): homozygous locus dropout.
2. No observed peaks, Q is heterozygous (D19): heterozygous locus dropout.
3. One observed peak, Q is heterozygous (D18): heterozygous single dropout.

Inserting a homozygous dropout peak of Q increases the WoE, which is further increased as the RFU of the peak increases (Figure 9, red).

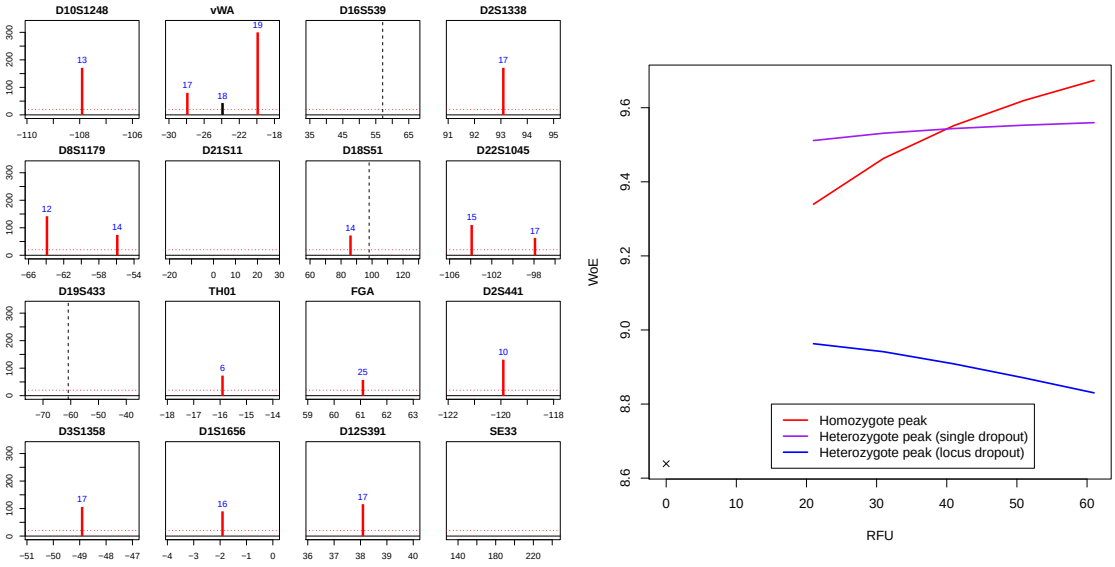
Inserting a heterozygous dropout peak of Q for which the corresponding allele was observed increases the WoE (Figure 9, purple) by more than when a homozygous allele was inserted, but the WoE increases less with increasing RFU of the inserted peak, so above 40 RFU the WoE is less with the inserted heterozygous peak than with the previously inserted homozygous peak. This is intuitive, as a small heterozygous peak is more likely than a small homozygous peak, leading to a greater WoE for the heterozygous peak at small RFUs. Similarly, a large heterozygous peak is less likely than a large homozygous peak, leading to a greater WoE for the homozygous peak at large RFUs.

Inserting a heterozygous dropout peak of Q for which the corresponding allele also dropped out increases the WoE initially (Figure 9, purple), but as the RFU of the peak is increased the WoE decreases. This is because the remaining dropout at this locus becomes less likely as the height of the artificial peak is increased; the variability in peak heights required to explain this observation increases with the increasing RFU of the introduced peak.

4.4.2 Altering observed peaks

A single observed peak in the CSP was given an altered RFU, from below the detection threshold (shown as 0 RFU here, analogous to dropout) to 150 RFU. This was performed for peaks at three separate loci with:

1. One observed peak, Q is homozygous (TH01): homozygous peak.



(a) CSP (b) Results

Figure 9: (a) The single-contributor CSP for which peak heights were altered. Vertical dashed lines indicate the position of dropped-out alleles that were inserted. (b) WoE for a single CSP when a dropped out allele is artificially inserted at differing RFUs.

2. One observed peak, Q is heterozygous (FGA): heterozygous peak with dropout.

3. Two observed peaks, Q is heterozygous (D22): heterozygous peak.

When the peak height of a homozygous peak of Q is altered, the WoE has a strong positive relationship with the RFU of the peak (Figure 10, red), while removing the peak entirely decreases the WoE substantially.

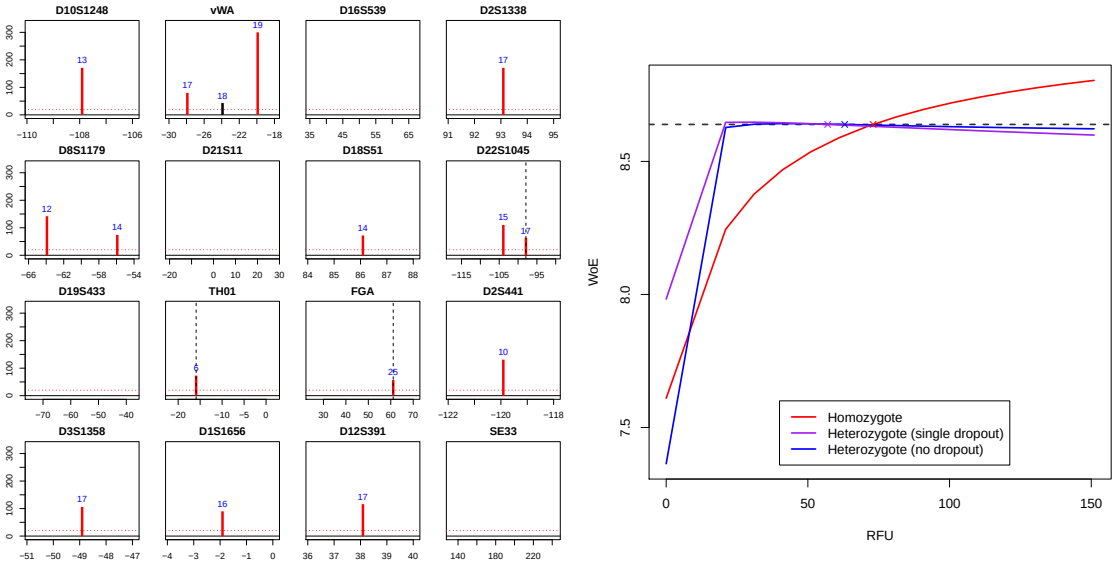
When the peak height of a heterozygous peak of Q for which the corresponding allele dropped out is altered, the WoE has a weak negative relationship with the RFU of the peak (Figure 10, purple), with a large decrease in WoE when the peak is removed entirely.

When the peak height of a heterozygous peak of Q for which the corresponding allele was also observed is altered, the WoE decreases slightly as the RFU of the peak deviates from that observed in the unaltered CSP (Figure 10, blue). Once again, removing the peak entirely decreases the WoE substantially.

Dropout of a heterozygote peak of Q for which the corresponding allele was observed is less likely than dropout of a heterozygous allele for which the corresponding allele has also dropped out (Figure 10, RFU=0, blue and purple), which make intuitive sense. However, dropout of a homozygous peak of Q is more likely than dropout of a heterozygote allele for which the corresponding allele has also dropped out (Figure 10, RFU=0, red and blue); this is counter-intuitive but results from the penalty on `scale` that `likeLTD` imposes, meaning the variance introduced under H_p by pairing a dropout peak with a non-dropout peak, which can be explained as a homozygous allele under H_d, is penalised greater than the dropout of a homozygous peak.

4.4.3 Dropin peak insertion

A single peak was inserted into the CSP at the six previously altered loci, with the newly inserted peak being at a non-Q allele, and so the inserted peak simulates a dropin event. At each of the six loci both the highest frequency non-Q allele and lowest frequency allele in the DNA17 NDU1 database (Caucasian) were



(a) CSP (b) Results

Figure 10: (a) The single-contributor CSP for which peak heights were altered. Vertical dashed lines indicate the position of altered peaks. (b) WoE for a single CSP when the peak heights of an observed peak is artificially altered, from 0 RFU to 151 RFU. Crosses and the dashed horizontal line indicate the WoE and RFU when no peak is altered.

inserted separately. Inserted alleles, and their associated population probabilities (without sampling or F_{ST} adjustment) are given in Table 5.

Locus	Common		Rare	
	Allele	Probability	Allele	Probability
D16S539	11	0.317	15	0.001
D18S51	12	0.149	8	0.000
D22S1045	16	0.369	19	0.001
D19S433	15	0.179	18	0.000
TH01	9.3	0.334	8.3	0.001
FGA	21	0.179	22.1	0.000

Table 5: Dropin alleles that were inserted into the donor 26 16pg DNA CSP. Common alleles were chosen as the highest frequency allele in the DNA17 NDU1 database not-shared with Q. Rare alleles were chosen as the lowest frequency allele in the database.

As expected, at all loci introducing a dropin peak decreases the WoE from the non-dropin WoE of 8.6 bans to between 7.0 and 8.5 bans (Figure 11). For all conditions the WoE is further reduced as the peak height of the dropin peak increases from 21 RFU to 61 RFU. The reduction in WoE varies substantially between dropin peaks at different loci, ranging from 0.05 bans at D22 with a 21 RFU dropin of a common allele to 1.6 bans at D19 with a 21 RFU dropin of a rare allele.

At D22 (red) both of the alleles of Q are observed in the CSP, plus the third introduced dropin peak. The WoE with introduction of a common (solid line) or rare (dashed line) allele diverge as the RFU of the introduced peak increases, because the dropin peak must be assigned as a dropin by `likeLTD` under H_p, which is plausible for a common allele, but implausible for a rare allele, and becomes increasingly implausible

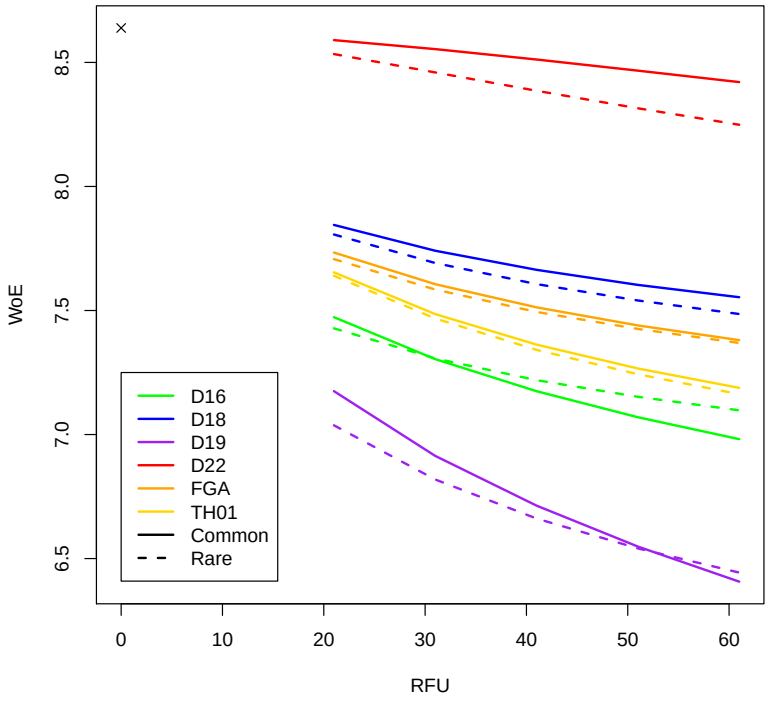


Figure 11: Weight-of-evidence for a single-contributor 16pg DNA CSP when a single rare or common dropin peak is inserted at one of six loci. See Table 5 for inserted alleles and their associated population probabilities.

as the RFU of the dropin peak increases.

At TH01 (yellow), FGA (orange) and D18 (blue), a single allelic peak (homozygous, heterozygous and heterozygous respectively) was observed in the CSP, plus the introduced dropin peak. At these loci, H_d explains the CSP as a heterozygous genotype composed of the observed true-allelic peak and the introduced dropin peak. Compared to the H_p explanation of a dropout and a dropin, this H_d explanation fits better when the dropin peak is rare than when it is common, leading to the WoE for the rare dropin being lower than that for the common dropin.

At D16 (green) and D19 (purple) no peaks were observed in the original CSP, so the CSPs here consist of just the introduced dropin peak. When the dropin peak is common in the population, under H_d `likeLTD` explains the observed peak as heterozygous at low RFU, but switches to explaining it as homozygous at high RFU. Conversely, when the dropin peak is very rare a homozygote is *a priori* unlikely, as under Hardy-Weinberg assumptions the probability of a homozygote is p_Z^2, which is 6.1e-7 and 1.5e-7 for the rare dropin allele at D16 and D19 respectively. For a common dropin the H_d explanation of a common homozygote allelic peak has an increasingly better likelihood compared to the H_p explanation of a common dropin as the RFU increases, leading to the reduction in WoE seen. However, for a rare dropin, the H_d explanation of a rare heterozygote peak does not increase its likelihood as much when the RFU increases, while the H_p explanation also performs less well as the RFU increases, so there is less discrepancy between the H_p and H_d explanations, leading to the lower drop in WoE seen in Figure 11.

4.4.4 Summary

We have demonstrated here that the peak height model behaves as expected when a CSP is altered in a number of ways; introducing a dropped out allele increases the WoE against Q, dropping out an allele decreases the WoE against Q and introducing a dropin peak decreased the WoE against Q. Increasing the RFU of homozygous Q alleles consistently increases the WoE against Q, as homozygous alleles are expected to be large. Increasing the RFU of heterozygous Q alleles has less of an effect on the WoE, but when the corresponding allele has dropped out the WoE decreases as a large observed peak with a dropout peak requires a large peak height variability to explain the CSP under H_p; these are often explained as homozygote peaks under H_d as would be expected for a large single peak. These are sensible and expected behaviours of the peak height model in response to altering the RFU of an allele contributed by Q. The WoE against Q decreases as the RFU of a dropin peak is increased, as would be expected, and the severity of the decrease in WoE when the dropin peak is introduced depends on the other observed peaks at the locus; if a dropin peak can only reasonably be explained as dropin it has little effect on the weight of evidence against Q, whereas if the dropin peak can be reasonably explained as an allele of X then the WoE against Q is significantly reduced. All of these behaviours are as expected.

4.5 Real case comparison: the Kercher murder case

A CSP from a real-world crime was evaluated with three continuous models. This comparison benchmarks the models against each other in a real-world scenario. Assuming that all of the models are valid, the results obtained with each should be similar.

4.5.1 Case circumstances

In November 2007, Meredith Kercher was murdered in her flat in Perugia, Italy. One man was tried and convicted for the crime with little controversy, but the accusation that Raffaele Sollecito and Amanda Knox were also involved in the murder was much more controversial. The two were found guilty in December 2009, acquitted in October 2011, found guilty again in January 2014, and finally ruled innocent by Italy's Supreme Court of Cassation in March 2015. One of the key, and controversial, pieces of evidence in the case against Ms Knox and Mr Sollecito was Ms Kercher's bra clasp, item 165B, found on the crime scene floor over a month after the murder occurred. Here, the WoE of the epg arising from the bra clasp for both Knox

Q	Sollecito			Knox		
Program	likeLTD	STRmix	EuroForMix	likeLTD	STRmix	EuroForMix
D8	0.7	0.2	0.8	-0.2	1.0	-0.2
D21	0.5	0.8	0.8	-0.1	0.1	0.1
D7	0.4	0.5	0.5	-0.2	-0.4	-0.1
CSF	0.7	0.3	0.6	-0.1	0.0	0.0
D3	0.8	0.8	1.0	-0.4	0.4	-0.2
TH01	1.1	0.8	1.2	-0.3	-0.6	-0.3
D13	0.7	0.7	0.8	0.0	-0.3	-0.1
D16	0.8	0.9	0.7	0.0	0.0	0.1
D2	2.4	1.6	2.1	0.1	0.5	0.0
D19	1.3	1.4	1.6	-0.9	-1.4	-1.4
vWA	1.5	1.9	1.8	-0.2	-0.7	-0.4
TPOX	0.9	0.7	0.7	-0.1	0.1	-0.1
D18	1.4	1.2	1.4	0.2	0.3	0.3
D5	-0.4	0.0	-0.5	0.0	0.3	0.1
FGA	-1.2	0.0	-0.5	0.0	0.1	0.0
Overall	11.5	11.8	13.0	-2.3	-0.7	-2.1

Table 6: Locus and overall weight of evidence (WoE) for the epg generated from item 165B (bra clasp) in the Kercher case. WoE was evaluated against Mr Sollecito or Ms Knox with three continuous models; `likeLTD`, `STRmix` and `EuroForMix`. In all evaluations Ms Kercher was assumed to be a contributor, with another unknown individual and Q/X. The IMP for Mr Sollecito is 18.5 bans. A detection threshold of 50 RFU was used in all evaluations.

and Sollecito to be a contributor will be evaluated using the `likeLTD`, `STRmix` and `EuroForMix` peak height models. The hypotheses compared are of the form:

$$H_p^S\text{: Q (Sollecito) + K1 (Kercher) + U1,}$$
$$H_d^S\text{: X + K1 (Kercher) + U1,}$$

and:

$$H_p^K\text{: Q (Knox) + K1 (Kercher) + U1,}$$
$$H_d^K\text{: X + K1 (Kercher) + U1.}$$

4.5.2 Results

When Q=Sollecito all three programs return a WoE$\geq$11.5 bans (Table 6) with `likeLTD` and `STRmix` having similar WoE ($\Delta = 0.3$ bans) but `EuroForMix` having a WoE > 1 ban larger (Δ=1.5 and 1.2 bans for `likeLTD` and `EuroForMix` respectively). The three programs have largely good correlation of the locus WoE, with two exceptions:

D5: `likeLTD` and `EuroForMix` have similar WoEs supporting H_d, `STRmix` supports neither hypothesis. Mr Sollecito is homozygous and masked by a heterozygous peak of Ms Kercher.

FGA: `likeLTD` and `EuroForMix` support H_d, `STRmix` supports neither hypothesis, the `likeLTD` and `EuroForMix` WoE are now considerably different. Mr Sollecito is heterozygous and both alleles are masked by alleles of Ms Kercher.

The runtime for `likeLTD` was between 16 and 17 minutes, while `EuroForMix` and `STRmix` took less than a minute to run.

When Q=Knox, all three programs support H_d (Table 6), with `likeLTD` and `EuroForMix` having similar WoE (≈-2 bans), while `STRmix` has a noticeably larger WoE (-0.7 bans). There are some notable locus differences between the programs:

D8: `EuroForMix` and `likeLTD` support H_d, `STRmix` has the strongest support for H_p of any program and any locus when querying Knox. Knox has one observed allele in a stutter position of an allele of Ms Kercher, and a dropout allele in the double-stutter position of the same allele.

D3: `EuroForMix` and `likeLTD` support H_d, `STRmix` supports H_p. Ms Knox has one allele masked, and another allele that has dropped out.

D13: `EuroForMix` and `STRmix` support H_d, `likeLTD` supports neither hypothesis. One allele of Knox is masked, while the other has dropped out.

The runtime for `likeLTD` was between 25 and 30 minutes, while `EuroForMix` and `STRmix` once again required less than a minute for computation.

5 Acknowledgements and version history

The underlying mathematical model and its implementation in the `likeLTD R` code were developed by DJB. Input into the model came from John Buckleton, as described in Balding and Buckleton (2009). A number of other academics and forensic scientists have given feedback and encouragement, among them Norah Rudin and Kirk Lohmueller in California, Torben Tvedebrink and Niels Morling in Denmark, Peter Gill (Norway), Hinda Haned (Netherlands), and Roberto Puch-Solis (UK).

Since Version 4.0, DJB has been helped to develop the `R` code by Adrian Timpson, and more recently Christopher Steele has developed and coded the continuous model and implemented the tests described in this document.

The early work in developing Version 5.0 was done by Adrian Timpson, the bulk of the recoding was done by Mayeul d'Avezac of the Research Software Development team in UCL Information Services Division, and some final enhancements were implemented by Christopher Steele.

The NIST databases added in Version 6.3 were collated into `likeLTD`format by Keith Inman, who has also given feedback and suggestions for features.

There has been no external funding for this project, although DJB has benefited from fees paid to UCL Consultants Ltd for expert witness work. His employer University College London, and in particular the UCL Genetics Institute, have supported the project by continuing to pay him a salary during the many months of work time that he has devoted to it.

- **Version 1**
  - Release 1-0, 19/1/10. The initial code had separate files LR1unk.R and LR2unk.R for 1 and 2 unprofiled contributors. Each included functions LRnumer() and LRdenom()
  - Release 1-1, 23/1/10. Restructured code for LR1unk.R to make it more similar to LR2unk.R
  - Release 1-2, 26/3/10. Fixed small bug reported by Kirk Lohmueller, affecting the assignment of allfracs in 3 places
  - Release 1-3, 24/5/10. Changed way dropin is modelled.
- **Version 2**
  - Release 2-0, 21/6/10. Merged previous LR1unk.R and LR2unk.R into a single file LTDNALR.R with the functions LRnumer() from those files renamed as LRnumer1() and LRnumer2(), respectively, and similarly for LRdenom().
  - Release 2-1. The change introduced in V2.1 has since been undone in V3.0, by introduction of a better way to deal with rare alleles

- **Version 3**
  - Release 3-0, 12/10/11. The previous functions LRnumer1(), LRnumer2(), LRdenom1() and LRdenom2() were all replaced by a single function `likeLTD`. There is now a distinct dropout rate for each replicate (DO). The dropout rate for other individuals is determined as a function of DO and the amount of DNA from that individual relative to the amount contributed by the reference individual (Q or U). We now strip out alleles with zero database frequency. If an allele of Q or CSP is not found in rownames(acbp) this allele is inserted into acbp with count 1. This has speeded up computations so that it now becomes feasible to allow three unprofiled contributors to the crime scene profile when Qcont=F, otherwise two unprofileds + Q. The model for dropout is now improved: the previous kdrop function has gone, and both dropout and dropin calculations are included in a new function Calclik(). Stutter alleles, or other apparent artefacts, can be entered as uncertain alleles allowing the possibility that they could be allelic.
  - Release 3-1, 4/1/12. Previously the dropin parameter DI was the non-dropout rate for a hypothetical extra individual, but this is now modified so that the dropin rate for each replicate is DI times the non-dropout rate (1-DO) for that replicate. As before, if DI=0 then all CSP alleles must come from one of the specified contributors. We now allow any of the profiled possible contributors to be unaffected by dropout, including Q. This option should only be used if the individual's alleles are observed in the CSP in every replicate at every locus; otherwise an error is generated. Alleles of profiled possible contributors not subject to dropout are converted to uncertain and removed from the CSP in the preprocessing step and (except for Q) don't play any further role in `likeLTD`. There has been some rearrangement of the code so that more work is done in a preprocessing function that is called only once, rather than being repeated in every call to the main function. Some changes have been made to the way parameters are named and passed; function calls to previous versions of `likeLTD` will not work without modification.
- **Version 4**
  - Release 4-0, 19/3/12. The main innovation is to allow dropout rates to increase with fragment length. Thus, fragment lengths for each allele in the profiling system being employed must be supplied (in base-pairs, bp, centred so that 0 represents an average length). These are passed to `likeLTD` in column 2 of matrix afbp, which replaces vector allfracs in Version 3.1; column 1 is the previous allfracs, and specifies population allele fractions. The program uses the model of Tvedebrink et al. (2012) and essentially the "dose" of DNA contributed by an individual at an allele is adjusted by a geometric function of fragment length (increased for below-average fragment lengths, and decreased for above-average). The rate of the geometric distribution is a parameter deg (for degradation), which is a vector with one entry per contributor subject to dropout.
  - Release 4-1, 8/5/12. Improvement to computation of number of simulations used when denNu=3 and also starting values for nupa and depa. Release of test document giving results from performance tests of `likeLTD`.
  - Release 4-2, 26/6/12. These are mainly minor changes to improve the output and program clarity documentation. The test results document distributed with this code is also updated to include new test results. The most important change is an improved assignment of the simulation size for the likelihood approximation invoked for three unprofiled contributors (i.e. denNu=3). For one or two unknown contributors there should be no changes to results from Version 4.1. BB is now passed as a parameter rather than being assigned as a constant.
  - Release 4-3, 10/8/12. Mostly just a few minor changes to documentation but there is one important bug fix that affected the likelihood calculations when DI > 0; thus any V4.2 runs that modelled dropin (Drin = TRUE in the wrapper) should be rerun with V4.3. Further improvements to output and to value for nsim.
  - Release 4-4, 2/11/12. Two changes:

* A new block of code can provide much faster computation when Nunp=2 or 3 and DI=0. The speed-up is greatest when the CSPs determine many alleles in the genotypes of the unprofiled contributors. The new code uses combinatorial functions that require the `R gtools` library; `library(gtools)` is now included in the Wrapper, but the package must first be installed using `install.packages("gtools")`. The result of the computation is unchanged from the original code that uses "for" loops. Both codes are kept, and the initial likelihood calculation is done once using each code in order to set flags indicating which is quickest; the faster code is then used for all subsequent calculations at that locus (there are separate flags for the calculations under H_p and H_d). Because of this improvement, the previous code that performed a simulation-based approximation to the likelihood when Nunp=3 has been removed, and so `nsim` has been removed from the list of parameters passed to `likeLTD`.
* Locus adjustment terms are now included in the dropout model, as in Tvedebrink et al. (2009). However, rather than estimate the locus effects on dropout from external data, they are estimated from the input data for the profile being analysed. Because this may be relatively little information, a strong prior is imposed on the locus adjustments: gamma with both parameters equal so that the mean is 1. The default value of this parameter (lap) is 50, giving a prior standard deviation for the locus adjustments of 0.14, the same as the SD of the estimates of Tvedebrink et al. (2009).

Also the inverse of the exact match probability is output for comparison with the LR for the observed CSP: this is the the standard match probability that would apply if the CSP showed exactly the reference profile of Q, and it is assumed that there is only one contributor. The LR for any other CSP should not exceed the inverse of the match probability.

– Release 4-5, 2/11/12. The power parameter β has been fixed in previous versions at -4.35 (Tvedebrink et al., 2009). In this version it is updated in the simulated annealing, separately under H_p and H_d, subject to a Gaussian prior/penalty with mean -4.35 and SD 0.38, the values obtained by Tvedebrink et al. (2009). This is a relatively minor and sensible change, and we have checked that it has little impact. However all the test results reported in this document are for V4-4 and not V4-5.

- **Version 5**

– Release 5-0. This is a complete re-writing of the basic code, which is now established as an official `R` package on CRAN. The simulated annealing algorithm used in previous versions for parameter optimisation is replaced with a differential evolution algorithm for optimisation. The underlying likelihood model remains the same as version 4.5, however, significant speed improvements have been gained through re-factoring of R code (e.g. converting for loops into vector/matrix operations), re-writing computationally intensive steps in C, and implementing parallel computation of the C code. Steps that have been implemented in C code include the computation of genotype combinations for unknown contributors, computing allele doses for each genotype combination, dose adjustments for relatedness, heterozygocity, dropout and power. Uploading the package to CRAN comes with improved documentation, version control and ease of access.

– Release 5-1. This update improved the calculation of the LR when close-relatedness is taken into account.

– Release 5-2. This update adds the function `get.likely.genotypes.peaks` that returns the most probable genotypes for each locus, and the most probable whole-profile genotype. There is an option to return marginal genotype probabilities for each contributor subject to dropout, or joint probabilities for all contributors subject to dropout.

– Release 5-3. This update improves the generation of both allele and output reports. These are now output as .doc files instead of .pdf files, and will now scale with the number of loci and the number of replicates correctly. The change to .doc files was motivated by client requests, and .pdf files can still be easily obtained by opening the .doc file in MS Word and saving as a pdf. There

are additional improvements to the checks for unusual alleles (which will now recognize typos and alleles not present in the database), and to the suggestion of appropriate hypotheses to test.

- Release 5-4. This update improves the optimisation procedure, replacing the simple convergence threshold with a geometric progression of convergence. This includes a geometric progression of the `DEoptim::DEoptim.control CR` variable, which controls the crossover rate of the optimisation algorithm. The combination of these two means that the parameter space is more thoroughly searched in the initial stages, leading to improved optimisation. L_p and L_d are now optimised together (within each step), allowing for estimation of the progress of optimisation (and an associated progress bar). Interim results after each step are now available. These changes are incorporated in the new optimisation function, `evaluate`. Small changes to the outputs are included, namely altered default file names (including the case name in the file name) and including which database file is used in the information section.
- Release 5-5. This update allows database alleles that are unobserved in both the CSP and reference profiles to be combined into a single "rare" allele, greatly improving the speed of computation. Three databases are now provided with `likeLTD`, for NGMSelect, SGM+ and Identifiler. The new default database is that for NGMSelect. A correction for linkage has been added, that will be utilised when Q and X are assumed to be siblings. The function `evaluate.from.interim` allows for a partial computation to be restarted from a generated interim result. The full posterior probability for genotypes can be returned, allowing for sensitivity testing of the LR to choices of alleles when a reference profile is only partially known.

- **Version 6**

  - Release 6-0. This major update introduces a new peak height model into `likeLTD`, which can utilise the full peak heights information available in a CSP, incorporating stutter, over-stutter, double-stutter, dropin, degradation, multiple replicates and multiple contributors. The peak height model can be run in a similar fashion to the discrete model, but with `.peaks` appended to each function e.g. `evaluate` becomes `evaluate.peaks`. The adjustment to the LR for linked loci has been extended to include uncle (or aunt)/nephew (or niece), half-uncle (or half-aunt)/half-nephew (or half niece), cousins, grandparent/grandchild and half siblings relationships. With this comes a new way of specifying relatedness, through an index of what relationship you wish to assume Q and X have, rather than the previous relatedness coefficients. This is applied to the discrete model as well as the peak height model. A seed to be set before running maximisation can now be handed to `evaluate` and `evaluate.peaks`, if unspecified an integer representation of the current date, time and process ID will be used. The seed used is now printed in the output report for both models.
  - Release 6-1. This release includes a substantial speed up of the program. Runtime for the Laboratory case has been reduced from approximately 363 and 2749 minutes for the 1U and 2U hypothesis pairs to 23 and 200 minutes. This was achieved by altering optimisation parameters: `nConverged` 5->4, `iterMax` 75->25, `searchPopFactor` 4->1. The function to determine the number of steps to run after the first was also altered to $\lceil (c+1)(r+1)\log_8 max(\sigma_p^2, \sigma_d^2) \rceil$ where c and r are the number of hypothesised contributors and the number of replicates in the CSP. An estimate of the contributions of unknown contributors was added to the allele report; this uses k-means clustering on the peak heights of unattributable alleles to assign each allele to an unknown contributor. If the estimated contribution of an unknown contributor is $< 1/3$ the estimated DNA contribution of Q then the allele report suggests that it may be possible to explain that unknown contributor as dropin rather than an extra unknown.
  - Release 6-2. This release includes changes to the allele report and output report, altering the presentation of CSP alleles and associated information. The estimation of Qs contribution used to suggest minors as dropin now only uses alleles of Q that are not shared with any K. Alleles that are below the specified detection threshold are now removed from the CSP automatically,

with a warning in the allele report. The default maximum dropin value has been changed from 100 to 250 RFU.

References

Balding, D. J. 2013. Evaluation of mixed-source, low-template dna profiles in forensic science. *Proc. Natn. Acad. Sci. USA*.

Balding, D. J. and Buckleton, J. 2009. Interpreting low template DNA profiles. *Forensic Sci. Int.-Gen.*, 4:1–10.

Balding, D. J. and Steele, C. D. 2015. *Weight-of-evidence for Forensic DNA Profiles*. John Wiley & Sons.

Bright, J.-A., Curran, J. M., and Buckleton, J. S. 2013a. Relatedness calculations for linked loci incorporating subpopulation effects. *Forensic Sci. Int.-Gen.*, 7(3):380–383.

Bright, J.-A., Taylor, D., Curran, J. M., and Buckleton, J. S. 2013b. Developing allelic and stutter peak height models for a continuous method of dna interpretation. *Forensic Sci. Int.-Gen.*, 7(2):296–304.

Brookes, C., Bright, J.-A., Harbison, S., and Buckleton, J. 2012. Characterising stutter in forensic str multiplexes. *Forensic Sci. Int.-Gen.*, 6(1):58–63.

Buckleton, J. S., Bright, J.-A., and Taylor, D. 2016. *Forensic DNA evidence interpretation*. CRC press.

Butler, J. 2010. *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press.

Cowell, R. G., Graversen, T., Lauritzen, S., and Mortera, J. 2013. Analysis of DNA Mixtures with Artefacts. *ArXiv e-prints*.

Gill, P., Whitaker, J., Flaxman, C., Brown, N., and Buckleton, J. 2000. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.*, 112:17–40.

Kelly, H., Bright, J.-A., Buckleton, J. S., and Curran, J. M. 2014. Identifying and modelling the drivers of stutter in forensic dna profiles. *Aust. J. Forensic Sci.*, 46(2):194–203.

Mullen, K. M., Ardia, D., Gil, D. L., Windover, D., Cline, J., et al. 2011. Deoptim: An r package for global optimization by differential evolution. *J. Stat. Softw.*, 40(6):1–26.

Steele, C. and Balding, D. 2016. Evaluation of low-template dna profiles using peak heights. *Stat. Appl. Genet. Mol. Biol.* In press.

Tvedebrink, T., Eriksen, P., Mogensen, H., and Morling, N. 2009. Estimating the probability of allelic drop-out of STR alleles in forensic genetics. *Forensic Sci. Int.-Gen.*, 3:222–226.

Tvedebrink, T., Eriksen, P., Mogensen, H., and Morling, N. 2012. Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out. *Forensic Sci. Int.-Gen.*, 6:97–101.

A Allele report for Example case

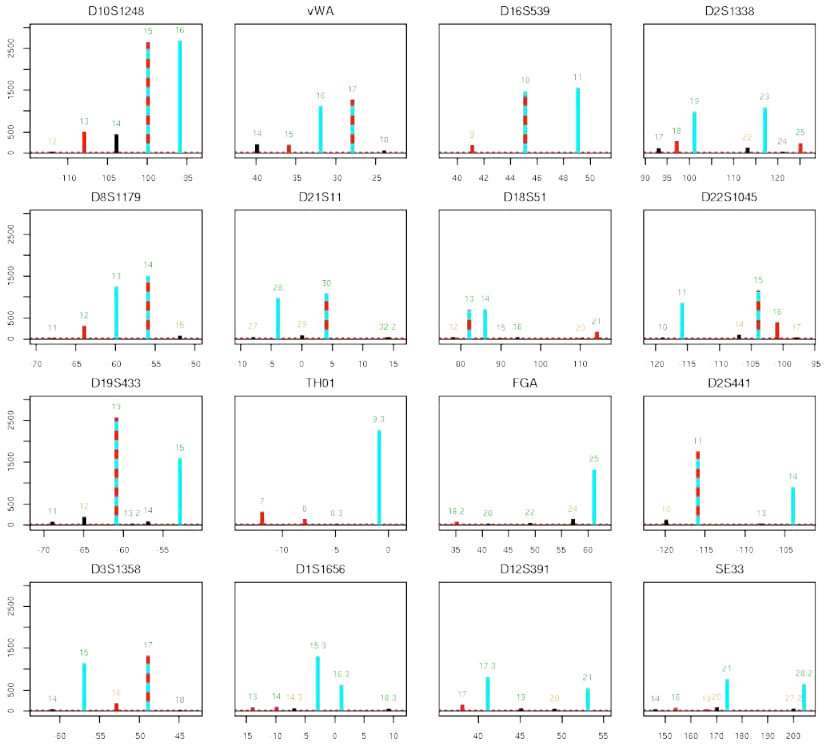
Example-Allele-Report

Example

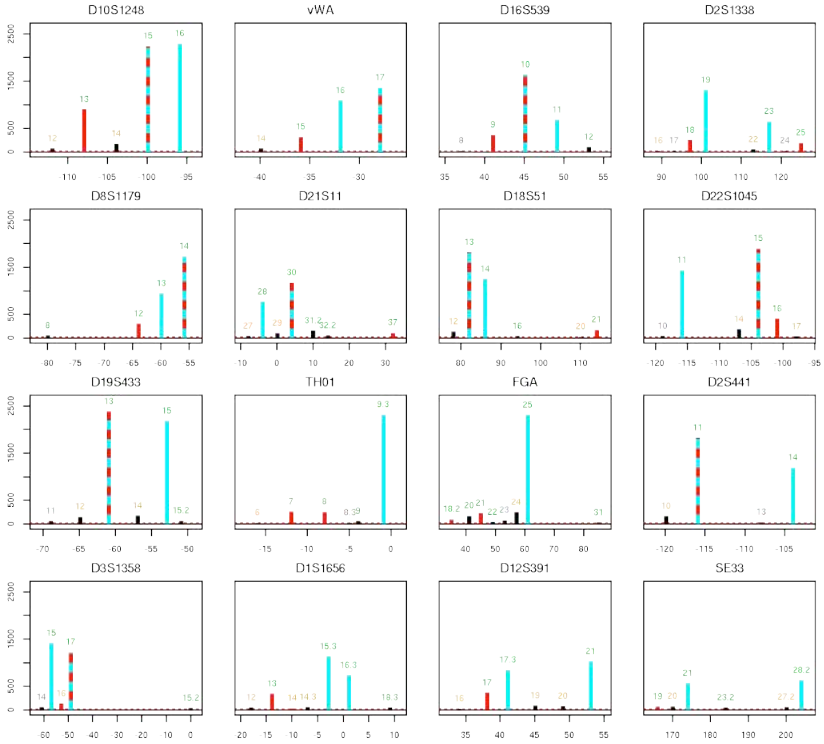
Label	Reference profile
Q	QUERIED
K1	KNOWN

Crime scene profiles (CSP)

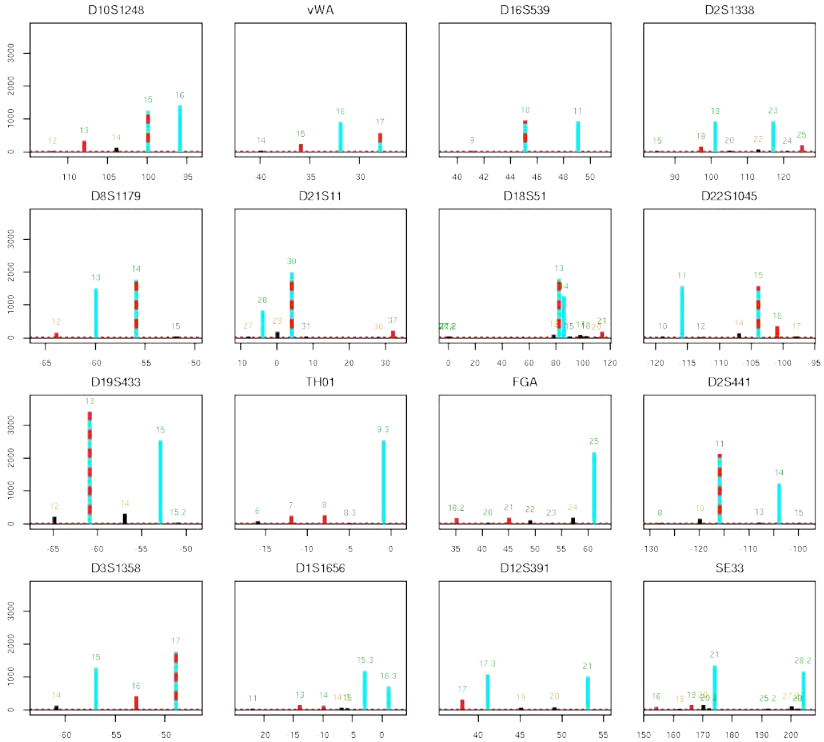
Replicate: exCSP.R1



Replicate: exCSP.R2



Replicate: exCSP.R3



The peak heights in RFU (y-axis) and mean adjusted allele length in base pairs (x-axis), with peaks at alleles in the profile of Q coloured in red, peaks at alleles of other assumed contributors shown with other colours, while black peaks are not attributable to Q or any other assumed contributor. Allele labels are coloured according to their possible allelic status (this is intended as a guide and is not assumed by the software): green=allelic, orange=uncertain, grey=non-allelic.

CSP alleles and peak heights

exCSP.R115

D10S1248					
Allele	12	13	14	15	16
Height	32	501	452	2656	2689
QUERIED	-	Het	-	Het	-
KNOWN	-	-	-	Het	Het

vWA					
Allele	14	15	16	17	18
Height	213	195	1120	1273	61
QUERIED	-	Het	-	Het	-
KNOWN	-	-	Het	Het	-

D16S539			
Allele	9	10	11
Height	183	1483	1564
QUERIED	Het	Het	-
KNOWN	-	Het	Het

D2S1338							
Allele	17	18	19	22	23	24	25
Height	121	278	990	123	1093	27	238
QUERIED	-	Het	-	-	-	-	Het
KNOWN	-	-	Het	-	Het	-	-

D8S1179					
Allele	11	12	13	14	15
Height	21	318	1256	1519	77
QUERIED	-	Het	-	Het	-
KNOWN	-	-	Het	Het	-

D21S11					
Allele	27	28	29	30	32.2
Height	50	978	96	1085	40
QUERIED	-	-	-	Het	-

D21S11					
KNOWN	-	Het	-	Het	-

D18S51							
Allele	12	13	14	15	16	20	21
Height	42	701	718	21	45	21	177
QUERIED	-	Het	-	-	-	-	Het
KNOWN	-	Het	Het	-	-	-	-

D22S1045						
Allele	10	11	14	15	16	17
Height	27	858	100	1156	397	27
QUERIED	-	-	-	Het	Het	-
KNOWN	-	Het	-	Het	-	-

D19S433						
Allele	11	12	13	13.2	14	15
Height	77	196	2575	33	97	1596
QUERIED	-	-	Hom	-	-	-
KNOWN	-	-	Het	-	-	Het

TH01				
Allele	7	8	8.3	9.3
Height	314	137	26	2279
QUERIED	Het	Het	-	-
KNOWN	-	-	-	Hom

FGA					
Allele	18.2	20	22	24	25
Height	75	28	50	140	1328
QUERIED	Het	-	-	-	-
KNOWN	-	-	-	-	Hom

D2S441				
Allele	10	11	13	14
Height	126	1764	33	905
QUERIED	-	Hom	-	-
KNOWN	-	Het	-	Het

D3S1358					
Allele	14	15	16	17	18
Height	39	1149	187	1327	21
QUERIED	-	-	Het	Het	-
KNOWN	-	Het	-	Het	-

D1S1656						
Allele	13	14	14.3	15.3	16.3	18.3
Height	90	102	71	1315	628	60
QUERIED	Het	Het	-	-	-	-
KNOWN	-	-	-	Het	Het	-

D12S391					
Allele	17	17.3	19	20	21
Height	146	819	72	53	555
QUERIED	Hom	-	-	-	-
KNOWN	-	Het	-	-	Het

SE33							
Allele	14	16	19	20	21	27.2	28.2
Height	43	82	42	87	766	57	650
QUERIED	-	Het	Het	-	-	-	-
KNOWN	-	-	-	-	Het	-	Het

exCSP.R215

D10S1248					
Allele	12	13	14	15	16
Height	66	902	169	2241	2292
QUERIED	-	Het	-	Het	-
KNOWN	-	-	-	Het	Het

vWA				
Allele	14	15	16	17
Height	66	315	1087	1358
QUERIED	-	Het	-	Het
KNOWN	-	-	Het	Het

D16S539					
Allele	8	9	10	11	12

D16S539					
Height	21	359	1637	681	99
QUERIED	-	Het	Het	-	-
KNOWN	-	-	Het	Het	-

D2S1338								
Allele	16	17	18	19	22	23	24	25
Height	21	21	250	1306	50	643	21	189
QUERIED	-	-	Het	-	-	-	-	Het
KNOWN	-	-	-	Het	-	Het	-	-

D8S1179				
Allele	8	12	13	14
Height	45	299	945	1726
QUERIED	-	Het	-	Het
KNOWN	-	-	Het	Het

D21S11							
Allele	27	28	29	30	31.2	32.2	37
Height	38	760	104	1169	150	45	105
QUERIED	-	-	-	Het	-	-	Het
KNOWN	-	Het	-	Het	-	-	-

D18S51						
Allele	12	13	14	16	20	21
Height	133	1812	1244	41	21	168
QUERIED	-	Het	-	-	-	Het
KNOWN	-	Het	Het	-	-	-

D22S1045						
Allele	10	11	14	15	16	17
Height	39	1432	186	1892	402	26
QUERIED	-	-	-	Het	Het	-
KNOWN	-	Het	-	Het	-	-

D19S433						
Allele	11	12	13	14	15	15.2
Height	57	147	2384	177	2186	63
QUERIED	-	-	Hom	-	-	-

D19S433						
KNOWN	-	-	Het	-	Het	-

TH01						
Allele	6	7	8	8.3	9	9.3
Height	21	260	253	21	62	2314
QUERIED	-	Het	Het	-	-	-
KNOWN	-	-	-	-	-	Hom

FGA								
Allele	18.2	20	21	22	23	24	25	31
Height	95	169	226	40	74	251	2310	29
QUERIED	Het	-	Het	-	-	-	-	-
KNOWN	-	-	-	-	-	-	Hom	-

D2S441				
Allele	10	11	13	14
Height	167	1824	32	1190
QUERIED	-	Hom	-	-
KNOWN	-	Het	-	Het

D3S1358					
Allele	14	15	15.2	16	17
Height	62	1418	34	135	1219
QUERIED	-	-	-	Het	Het
KNOWN	-	Het	-	-	Het

D1S1656							
Allele	12	13	14	14.3	15.3	16.3	18.3
Height	49	340	33	58	1132	732	46
QUERIED	-	Het	Het	-	-	-	-
KNOWN	-	-	-	-	Het	Het	-

D12S391						
Allele	16	17	17.3	19	20	21
Height	21	362	842	90	81	1023
QUERIED	-	Hom	-	-	-	-
KNOWN	-	-	Het	-	-	Het

SE33						
Allele	19	20	21	23.2	27.2	28.2
Height	68	74	566	46	55	630
QUERIED	Het	-	-	-	-	-
KNOWN	-	-	Het	-	-	Het

exCSP.R315

D10S1248					
Allele	12	13	14	15	16
Height	21	338	134	1268	1414
QUERIED	-	Het	-	Het	-
KNOWN	-	-	-	Het	Het

vWA				
Allele	14	15	16	17
Height	34	236	912	589
QUERIED	-	Het	-	Het
KNOWN	-	-	Het	Het

D16S539			
Allele	9	10	11
Height	39	956	925
QUERIED	Het	Het	-
KNOWN	-	Het	Het

D2S1338								
Allele	15	18	19	20	22	23	24	25
Height	29	158	926	36	70	944	21	200
QUERIED	-	Het	-	-	-	-	-	Het
KNOWN	-	-	Het	-	-	Het	-	-

D8S1179				
Allele	12	13	14	15
Height	167	1509	1779	41
QUERIED	Het	-	Het	-
KNOWN	-	Het	Het	-

D21S11							
Allele	27	28	29	30	31	36	37

D21S11							
Height	46	842	196	2005	37	21	219
QUERIED	-	-	-	Het	-	-	Het
KNOWN	-	Het	-	Het	-	-	-

D18S51											
Allele	7.2	12	13	14	15	17	17.2	18	20	21	21.2
Height	25	105	1798	1262	36	91	30	57	21	198	37
QUERIED	-	-	Het	-	-	-	-	-	-	Het	-
KNOWN	-	-	Het	Het	-	-	-	-	-	-	-

D22S1045							
Allele	10	11	12	14	15	16	17
Height	41	1570	33	146	1581	352	35
QUERIED	-	-	-	-	Het	Het	-
KNOWN	-	Het	-	-	Het	-	-

D19S433					
Allele	12	13	14	15	15.2
Height	216	3416	311	2535	35
QUERIED	-	Hom	-	-	-
KNOWN	-	Het	-	Het	-

TH01					
Allele	6	7	8	8.3	9.3
Height	79	250	260	21	2549
QUERIED	-	Het	Het	-	-
KNOWN	-	-	-	-	Hom

FGA							
Allele	18.2	20	21	22	23	24	25
Height	172	39	194	122	21	185	2184
QUERIED	Het	-	Het	-	-	-	-
KNOWN	-	-	-	-	-	-	Hom

D2S441						
Allele	8	10	11	13	14	15
Height	26	162	2120	46	1235	26
QUERIED	-	-	Hom	-	-	-

D2S441						
KNOWN	-	-	Het	-	Het	-

D3S1358				
Allele	14	15	16	17
Height	129	1289	411	1780
QUERIED	-	-	Het	Het
KNOWN	-	Het	-	Het

D1S1656							
Allele	11	13	14	14.3	15	15.3	16.3
Height	44	143	131	70	59	1192	721
QUERIED	-	Het	Het	-	-	-	-
KNOWN	-	-	-	-	-	Het	Het

D12S391					
Allele	17	17.3	19	20	21
Height	315	1085	70	93	1021
QUERIED	Hom	-	-	-	-
KNOWN	-	Het	-	-	Het

SE33										
Allele	16	18	19	20	20.2	21	25.2	27.2	28	28.2
Height	97	21	142	141	49	1357	41	117	38	1178
QUERIED	Het	-	Het	-	-	-	-	-	-	-
KNOWN	-	-	-	-	-	Het	-	-	-	Het

Summary

Reference profile	QUERIED	KNOWN
Replicate: exCSP.R1	0.9375	1
Replicate: exCSP.R2	0.96875	1
Replicate: exCSP.R3	1	1
Overall	0.96875	1

Approximate representation (observed/total) for each reference profile per replicate and overall.

Alleles that are rare in at least one database

Locus	File	Profile	Allele	Issue	NDU1	NDU2	NDU3	NDU4	NDU6	NDU7
D2S1338	CSP	exCSP.R2	16	Rare allele	113	48	1	3	23	25
D2S1338	CSP	exCSP.R3	15	Rare allele	0	1	0	0	1	0
D8S1179	CSP	exCSP.R2	8	Rare allele	42	0	3	0	0	0
D21S11	CSP	exCSP.R1	27	Rare allele	92	49	4	0	25	24
D21S11	CSP	exCSP.R2	27	Rare allele	92	49	4	0	25	24
D21S11	CSP	exCSP.R2	37	Rare allele	0	1	0	0	0	1
D21S11	CSP	exCSP.R3	27	Rare allele	92	49	4	0	25	24
D21S11	CSP	exCSP.R3	36	Rare allele	0	6	0	0	1	5
D21S11	CSP	exCSP.R3	37	Rare allele	0	1	0	0	0	1
D21S11	Reference	QUERIED	37	Rare allele	0	1	0	0	0	1
D18S51	CSP	exCSP.R3	7.2	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
D18S51	CSP	exCSP.R3	17.2	Rare allele	0	2	0	0	2	0
D18S51	CSP	exCSP.R3	21.2	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
D22S1045	CSP	exCSP.R1	10	Rare allele	0	34	1	0	20	14
D22S1045	CSP	exCSP.R2	10	Rare allele	0	34	1	0	20	14
D22S1045	CSP	exCSP.R3	10	Rare allele	0	34	1	0	20	14
D22S1045	CSP	exCSP.R3	12	Rare allele	22	42	0	1	26	16
D19S433	CSP	exCSP.R1	11	Rare allele	9	66	0	0	37	29
D19S433	CSP	exCSP.R2	11	Rare allele	9	66	0	0	37	29
TH01	CSP	exCSP.R1	8.3	Rare allele	3	0	1	0	0	0
TH01	CSP	exCSP.R2	8.3	Rare allele	3	0	1	0	0	0
TH01	CSP	exCSP.R3	8.3	Rare allele	3	0	1	0	0	0
FGA	CSP	exCSP.R1	18.2	Rare allele	0	15	0	0	10	5
FGA	CSP	exCSP.R2	18.2	Rare allele	0	15	0	0	10	5
FGA	CSP	exCSP.R2	31	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
FGA	CSP	exCSP.R3	18.2	Rare allele	0	15	0	0	10	5
FGA	Reference	QUERIED	18.2	Rare allele	0	15	0	0	10	5
D2S441	CSP	exCSP.R3	8	Rare allele	5	0	1	0	0	0
D3S1358	CSP	exCSP.R2	15.2	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
D1S1656	CSP	exCSP.R1	14.3	Rare allele	7	2	0	0	0	2
D1S1656	CSP	exCSP.R1	15.3	Rare allele	169	13	8	0	5	8
D1S1656	CSP	exCSP.R2	14.3	Rare allele	7	2	0	0	0	2
D1S1656	CSP	exCSP.R2	15.3	Rare allele	169	13	8	0	5	8
D1S1656	CSP	exCSP.R3	14.3	Rare allele	7	2	0	0	0	2
D1S1656	CSP	exCSP.R3	15.3	Rare allele	169	13	8	0	5	8
D1S1656	Reference	KNOWN	15.3	Rare allele	169	13	8	0	5	8
D12S391	CSP	exCSP.R1	17.3	Rare allele	71	0	0	0	0	0
D12S391	CSP	exCSP.R2	16	Rare allele	72	58	3	1	24	34
D12S391	CSP	exCSP.R2	17.3	Rare allele	71	0	0	0	0	0
D12S391	CSP	exCSP.R3	17.3	Rare allele	71	0	0	0	0	0
D12S391	Reference	KNOWN	17.3	Rare allele	71	0	0	0	0	0
SE33	CSP	exCSP.R2	23.2	Rare allele	92	5	8	19	1	4
SE33	CSP	exCSP.R3	20.2	Rare allele	36	2	3	5	0	2
SE33	CSP	exCSP.R3	28	Rare allele	0	0	0	0	0	0

Suggested parameter values

nU	doDropin	Guidance
3	No	Can only be evaluated by removing the additional U from defence
2	Yes	Inefficient approximation
1	Yes	Estimated U if dropin modelled

If an nU value >2 is indicated, an approximate result can be obtained using nU=2 and doDropin=Yes. Please check the allele designations shown in the CSP plots that were used to generate these hypotheses; if you disagree with the suggested designations the recommendations here may need to be altered.

Minor as dropin

Mean RFU Q	Mean RFU U1	Mean RFU U2	estimated # U required with dropin
204	45	148	1

Mean peak height for Q, clustered mean peak heights for unknowns using k-means clustering with 2 clusters, and the minimum number of unknowns required to explain the CSP with dropin (mean Q peak height/mean U peak height ≤ 3).

System information

Type	Details
Date report generated:	Fri Feb 9 16:34:50 2018
Package	likeLTD
Title	Tools to Evaluate DNA Profile Evidence
Description	Tools to determine DNA profile Weight of Evidence. For further information see the 'likeLTD' guide provided, Balding, D.J. (2013) <DOI:10.1073/pnas.1219739110>, or Steele, C.D. et al. (2016) <DOI:10.1515/sagmb-2016-0038>.
Depends	R (≥ 2.10), DEoptim, ggplot2, gtools, rtf
Suggests	svUnit, scales
Imports	gdata, tools, tcltk
Version	6.3.0
Date	2017-06-11
Author	David Balding, Adrian Timpson, Christopher Steele, Mayeul d'Avezac, James Hetherington.
Maintainer	Christopher Steele <c.steele.11@ucl.ac.uk>
License	GPL-3
URL	https://sites.google.com/site/baldingstatisticalgenetics/
NeedsCompilation	yes
Packaged	2018-02-09 16:17:20 UTC; chris
Built	R 3.3.1; x86_64-pc-linux-gnu; 2018-02-09 16:17:20 UTC; unix
sysname	Linux
release	4.4.0-109-generic
version	#132-Ubuntu SMP Tue Jan 9 19:52:39 UTC 2018
nodename	chris-OptiPlex-9010
machine	x86_64
login	unknown
user	chris
effective_user	chris

B Output file for Example case

Example-Evaluation-Report
Example

Prosecution hypothesis: QUERIED (Q) + KNOWN (K1) + U1 + dropin

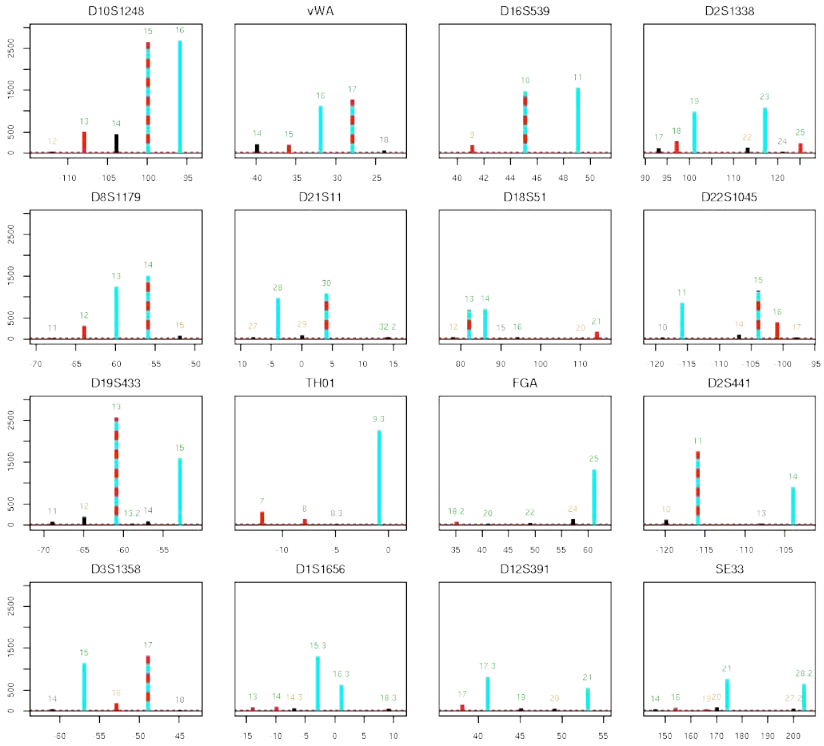
Defence hypothesis: Unknown (X) + KNOWN (K1) + U1 + dropin

Overall Likelihood

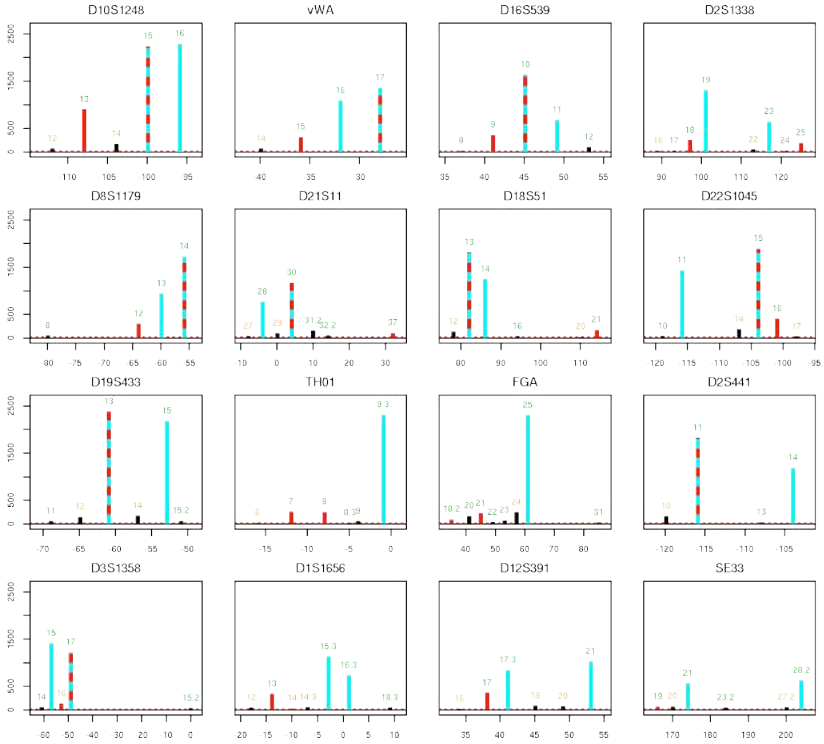
calculation	estimate
Prosecution.log10	-829.8
Defence.log10	-847.8
Ratio.log10	18.0
Ratio	1.1e+18

Crime scene profiles (CSP)

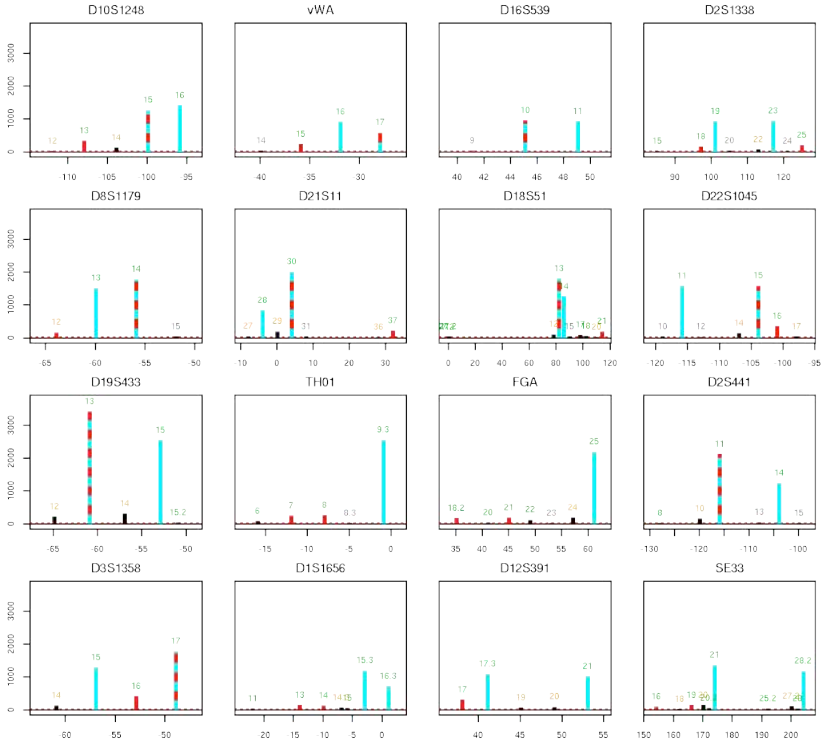
Replicate: exCSP.R1



Replicate: exCSP.R2



Replicate: exCSP.R3



The peak heights in RFU (y-axis) and mean adjusted allele length in base pairs (x-axis), with peaks at alleles in the profile of Q coloured in red, peaks at alleles of other assumed contributors shown with other colours, while black peaks are not attributable to Q or any other assumed contributor. Allele labels are coloured according to their possible allelic status (this is intended as a guide and is not assumed by the software): green=allelic, orange=uncertain, grey=non-allelic.

CSP alleles and peak heights

exCSP.R115

D10S1248					
Allele	12	13	14	15	16
Height	32	501	452	2656	2689
KNOWN	-	-	-	Het	Het
QUERIED	-	Het	-	Het	-

vWA					
Allele	14	15	16	17	18
Height	213	195	1120	1273	61
KNOWN	-	-	Het	Het	-
QUERIED	-	Het	-	Het	-

D16S539			
Allele	9	10	11
Height	183	1483	1564
KNOWN	-	Het	Het
QUERIED	Het	Het	-

D2S1338							
Allele	17	18	19	22	23	24	25
Height	121	278	990	123	1093	27	238
KNOWN	-	-	Het	-	Het	-	-
QUERIED	-	Het	-	-	-	-	Het

D8S1179					
Allele	11	12	13	14	15
Height	21	318	1256	1519	77
KNOWN	-	-	Het	Het	-
QUERIED	-	Het	-	Het	-

D21S11					
Allele	27	28	29	30	32.2
Height	50	978	96	1085	40
KNOWN	-	Het	-	Het	-

D21S11						
QUERIED	-	-	-	Het	-	

D18S51							
Allele	12	13	14	15	16	20	21
Height	42	701	718	21	45	21	177
KNOWN	-	Het	Het	-	-	-	-
QUERIED	-	Het	-	-	-	-	Het

D22S1045						
Allele	10	11	14	15	16	17
Height	27	858	100	1156	397	27
KNOWN	-	Het	-	Het	-	-
QUERIED	-	-	-	Het	Het	-

D19S433						
Allele	11	12	13	13.2	14	15
Height	77	196	2575	33	97	1596
KNOWN	-	-	Het	-	-	Het
QUERIED	-	-	Hom	-	-	-

TH01				
Allele	7	8	8.3	9.3
Height	314	137	26	2279
KNOWN	-	-	-	Hom
QUERIED	Het	Het	-	-

FGA					
Allele	18.2	20	22	24	25
Height	75	28	50	140	1328
KNOWN	-	-	-	-	Hom
QUERIED	Het	-	-	-	-

D2S441				
Allele	10	11	13	14
Height	126	1764	33	905
KNOWN	-	Het	-	Het
QUERIED	-	Hom	-	-

D3S1358					
Allele	14	15	16	17	18
Height	39	1149	187	1327	21
KNOWN	-	Het	-	Het	-
QUERIED	-	-	Het	Het	-

D1S1656						
Allele	13	14	14.3	15.3	16.3	18.3
Height	90	102	71	1315	628	60
KNOWN	-	-	-	Het	Het	-
QUERIED	Het	Het	-	-	-	-

D12S391					
Allele	17	17.3	19	20	21
Height	146	819	72	53	555
KNOWN	-	Het	-	-	Het
QUERIED	Hom	-	-	-	-

SE33							
Allele	14	16	19	20	21	27.2	28.2
Height	43	82	42	87	766	57	650
KNOWN	-	-	-	-	Het	-	Het
QUERIED	-	Het	Het	-	-	-	-

exCSP.R215

D10S1248					
Allele	12	13	14	15	16
Height	66	902	169	2241	2292
KNOWN	-	-	-	Het	Het
QUERIED	-	Het	-	Het	-

vWA				
Allele	14	15	16	17
Height	66	315	1087	1358
KNOWN	-	-	Het	Het
QUERIED	-	Het	-	Het

D16S539					
Allele	8	9	10	11	12

D16S539					
Height	21	359	1637	681	99
KNOWN	-	-	Het	Het	-
QUERIED	-	Het	Het	-	-

D2S1338								
Allele	16	17	18	19	22	23	24	25
Height	21	21	250	1306	50	643	21	189
KNOWN	-	-	-	Het	-	Het	-	-
QUERIED	-	-	Het	-	-	-	-	Het

D8S1179				
Allele	8	12	13	14
Height	45	299	945	1726
KNOWN	-	-	Het	Het
QUERIED	-	Het	-	Het

D21S11							
Allele	27	28	29	30	31.2	32.2	37
Height	38	760	104	1169	150	45	105
KNOWN	-	Het	-	Het	-	-	-
QUERIED	-	-	-	Het	-	-	Het

D18S51						
Allele	12	13	14	16	20	21
Height	133	1812	1244	41	21	168
KNOWN	-	Het	Het	-	-	-
QUERIED	-	Het	-	-	-	Het

D22S1045						
Allele	10	11	14	15	16	17
Height	39	1432	186	1892	402	26
KNOWN	-	Het	-	Het	-	-
QUERIED	-	-	-	Het	Het	-

D19S433						
Allele	11	12	13	14	15	15.2
Height	57	147	2384	177	2186	63
KNOWN	-	-	Het	-	Het	-

D19S433						
QUERIED	-	-	Hom	-	-	-

TH01						
Allele	6	7	8	8.3	9	9.3
Height	21	260	253	21	62	2314
KNOWN	-	-	-	-	-	Hom
QUERIED	-	Het	Het	-	-	-

FGA								
Allele	18.2	20	21	22	23	24	25	31
Height	95	169	226	40	74	251	2310	29
KNOWN	-	-	-	-	-	-	Hom	-
QUERIED	Het	-	Het	-	-	-	-	-

D2S441				
Allele	10	11	13	14
Height	167	1824	32	1190
KNOWN	-	Het	-	Het
QUERIED	-	Hom	-	-

D3S1358					
Allele	14	15	15.2	16	17
Height	62	1418	34	135	1219
KNOWN	-	Het	-	-	Het
QUERIED	-	-	-	Het	Het

D1S1656							
Allele	12	13	14	14.3	15.3	16.3	18.3
Height	49	340	33	58	1132	732	46
KNOWN	-	-	-	-	Het	Het	-
QUERIED	-	Het	Het	-	-	-	-

D12S391						
Allele	16	17	17.3	19	20	21
Height	21	362	842	90	81	1023
KNOWN	-	-	Het	-	-	Het
QUERIED	-	Hom	-	-	-	-

SE33						
Allele	19	20	21	23.2	27.2	28.2
Height	68	74	566	46	55	630
KNOWN	-	-	Het	-	-	Het
QUERIED	Het	-	-	-	-	-

exCSP.R315

D10S1248					
Allele	12	13	14	15	16
Height	21	338	134	1268	1414
KNOWN	-	-	-	Het	Het
QUERIED	-	Het	-	Het	-

vWA				
Allele	14	15	16	17
Height	34	236	912	589
KNOWN	-	-	Het	Het
QUERIED	-	Het	-	Het

D16S539			
Allele	9	10	11
Height	39	956	925
KNOWN	-	Het	Het
QUERIED	Het	Het	-

D2S1338								
Allele	15	18	19	20	22	23	24	25
Height	29	158	926	36	70	944	21	200
KNOWN	-	-	Het	-	-	Het	-	-
QUERIED	-	Het	-	-	-	-	-	Het

D8S1179				
Allele	12	13	14	15
Height	167	1509	1779	41
KNOWN	-	Het	Het	-
QUERIED	Het	-	Het	-

D21S11							
Allele	27	28	29	30	31	36	37

D21S11							
Height	46	842	196	2005	37	21	219
KNOWN	-	Het	-	Het	-	-	-
QUERIED	-	-	-	Het	-	-	Het

D18S51											
Allele	7.2	12	13	14	15	17	17.2	18	20	21	21.2
Height	25	105	1798	1262	36	91	30	57	21	198	37
KNOWN	-	-	Het	Het	-	-	-	-	-	-	-
QUERIED	-	-	Het	-	-	-	-	-	-	Het	-

D22S1045							
Allele	10	11	12	14	15	16	17
Height	41	1570	33	146	1581	352	35
KNOWN	-	Het	-	-	Het	-	-
QUERIED	-	-	-	-	Het	Het	-

D19S433					
Allele	12	13	14	15	15.2
Height	216	3416	311	2535	35
KNOWN	-	Het	-	Het	-
QUERIED	-	Hom	-	-	-

TH01					
Allele	6	7	8	8.3	9.3
Height	79	250	260	21	2549
KNOWN	-	-	-	-	Hom
QUERIED	-	Het	Het	-	-

FGA							
Allele	18.2	20	21	22	23	24	25
Height	172	39	194	122	21	185	2184
KNOWN	-	-	-	-	-	-	Hom
QUERIED	Het	-	Het	-	-	-	-

D2S441						
Allele	8	10	11	13	14	15
Height	26	162	2120	46	1235	26
KNOWN	-	-	Het	-	Het	-

D2S441						
QUERIED	-	-	Hom	-	-	-

D3S1358				
Allele	14	15	16	17
Height	129	1289	411	1780
KNOWN	-	Het	-	Het
QUERIED	-	-	Het	Het

D1S1656							
Allele	11	13	14	14.3	15	15.3	16.3
Height	44	143	131	70	59	1192	721
KNOWN	-	-	-	-	-	Het	Het
QUERIED	-	Het	Het	-	-	-	-

D12S391					
Allele	17	17.3	19	20	21
Height	315	1085	70	93	1021
KNOWN	-	Het	-	-	Het
QUERIED	Hom	-	-	-	-

SE33										
Allele	16	18	19	20	20.2	21	25.2	27.2	28	28.2
Height	97	21	142	141	49	1357	41	117	38	1178
KNOWN	-	-	-	-	-	Het	-	-	-	Het
QUERIED	Het	-	Het	-	-	-	-	-	-	-

Summary

Reference profile	KNOWN	QUERIED
Replicate: exCSP.R1	1	0.9375
Replicate: exCSP.R2	1	0.96875
Replicate: exCSP.R3	1	1
Overall	1	0.96875

Approximate representation (observed/total) for each reference profile per replicate and overall.

Alleles that are rare in at least one database

Locus	File	Profile	Allele	Issue	NDU1	NDU2	NDU3	NDU4	NDU6	NDU7
D2S1338	CSP	exCSP.R2	16	Rare allele	113	48	1	3	23	25
D2S1338	CSP	exCSP.R3	15	Rare allele	0	1	0	0	1	0
D8S1179	CSP	exCSP.R2	8	Rare allele	42	0	3	0	0	0
D21S11	CSP	exCSP.R1	27	Rare allele	92	49	4	0	25	24
D21S11	CSP	exCSP.R2	27	Rare allele	92	49	4	0	25	24
D21S11	CSP	exCSP.R2	37	Rare allele	0	1	0	0	0	1
D21S11	CSP	exCSP.R3	27	Rare allele	92	49	4	0	25	24
D21S11	CSP	exCSP.R3	36	Rare allele	0	6	0	0	1	5
D21S11	CSP	exCSP.R3	37	Rare allele	0	1	0	0	0	1
D21S11	Reference	QUERIED	37	Rare allele	0	1	0	0	0	1
D18S51	CSP	exCSP.R3	7.2	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
D18S51	CSP	exCSP.R3	17.2	Rare allele	0	2	0	0	2	0
D18S51	CSP	exCSP.R3	21.2	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
D22S1045	CSP	exCSP.R1	10	Rare allele	0	34	1	0	20	14
D22S1045	CSP	exCSP.R2	10	Rare allele	0	34	1	0	20	14
D22S1045	CSP	exCSP.R3	10	Rare allele	0	34	1	0	20	14
D22S1045	CSP	exCSP.R3	12	Rare allele	22	42	0	1	26	16
D19S433	CSP	exCSP.R1	11	Rare allele	9	66	0	0	37	29
D19S433	CSP	exCSP.R2	11	Rare allele	9	66	0	0	37	29
TH01	CSP	exCSP.R1	8.3	Rare allele	3	0	1	0	0	0
TH01	CSP	exCSP.R2	8.3	Rare allele	3	0	1	0	0	0
TH01	CSP	exCSP.R3	8.3	Rare allele	3	0	1	0	0	0
FGA	CSP	exCSP.R1	18.2	Rare allele	0	15	0	0	10	5
FGA	CSP	exCSP.R2	18.2	Rare allele	0	15	0	0	10	5
FGA	CSP	exCSP.R2	31	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
FGA	CSP	exCSP.R3	18.2	Rare allele	0	15	0	0	10	5
FGA	Reference	QUERIED	18.2	Rare allele	0	15	0	0	10	5
D2S441	CSP	exCSP.R3	8	Rare allele	5	0	1	0	0	0
D3S1358	CSP	exCSP.R2	15.2	Not in database	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)	integer(0)
D1S1656	CSP	exCSP.R1	14.3	Rare allele	7	2	0	0	0	2
D1S1656	CSP	exCSP.R1	15.3	Rare allele	169	13	8	0	5	8
D1S1656	CSP	exCSP.R2	14.3	Rare allele	7	2	0	0	0	2
D1S1656	CSP	exCSP.R2	15.3	Rare allele	169	13	8	0	5	8
D1S1656	CSP	exCSP.R3	14.3	Rare allele	7	2	0	0	0	2
D1S1656	CSP	exCSP.R3	15.3	Rare allele	169	13	8	0	5	8
D1S1656	Reference	KNOWN	15.3	Rare allele	169	13	8	0	5	8
D12S391	CSP	exCSP.R1	17.3	Rare allele	71	0	0	0	0	0
D12S391	CSP	exCSP.R2	16	Rare allele	72	58	3	1	24	34
D12S391	CSP	exCSP.R2	17.3	Rare allele	71	0	0	0	0	0
D12S391	CSP	exCSP.R3	17.3	Rare allele	71	0	0	0	0	0
D12S391	Reference	KNOWN	17.3	Rare allele	71	0	0	0	0	0
SE33	CSP	exCSP.R2	23.2	Rare allele	92	5	8	19	1	4
SE33	CSP	exCSP.R3	20.2	Rare allele	36	2	3	5	0	2
SE33	CSP	exCSP.R3	28	Rare allele	0	0	0	0	0	0

Likelihoods at each locus

Likelihood	D10S1248	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D22S1045	D19S433	TH01	FGA	D2S441	D3S1358	D1S1656	D12S391	SE33
Prosecution.log10	-52.19	-42.01	-36.92	-63.40	-39.91	-54.96	-72.67	-51.18	-60.42	-40.17	-57.75	-40.61	-42.22	-58.79	-47.94	-68.60
Defence.log10	-52.08	-42.82	-38.03	-64.81	-41.10	-56.62	-74.49	-51.77	-61.32	-41.56	-59.34	-41.49	-42.79	-60.32	-49.11	-70.12
Ratio.log10	-0.11	0.81	1.10	1.41	1.19	1.67	1.82	0.60	0.90	1.39	1.59	0.88	0.57	1.53	1.17	1.52
Ratio	0.78	6.47	12.65	25.91	15.43	46.31	66.78	3.95	7.97	24.80	38.63	7.54	3.73	33.80	14.83	32.76

Theoretical maximum LR = Inverse Match Probability (IMP)

calculation	estimate
likelihood ratio	1.23e+22
Log10 likelihood ratio	22.1

DNA contribution (RFU) and degradation estimates

Prosecution	U1	KNOWN	QUERIED
Replicate: exCSP.R1	21.44	1091.85	169.51
Replicate: exCSP.R2	24.11	1228.27	190.69
Replicate: exCSP.R3	24.73	1259.68	195.57
Degradation	0	0.00164	0.00422
Defence	**U1**	**X**	**KNOWN**
Replicate: exCSP.R1	169.04	24.85	1087.57
Replicate: exCSP.R2	189.42	27.84	1218.67
Replicate: exCSP.R3	194.41	28.58	1250.76
Degradation	0.00548	0	0.00154

Dropin parameter estimates

hypothesis	dropin

hypothesis	dropin
Prosecution	183.800
Defence	185.049

User defined parameters

Parameter	User input
nUnknowns	1
ethnic	NDU1
adj	1
fst	0.03
relatedness1	0
relatedness2	0
relationship	0
doDropin	Yes
doDoubleStutter	Yes
doOverStutter	Yes
detectionThresh	20

Input files

File	Used
CSP	exampleCSP.csv
Reference	exampleRef.csv
Database	DNA17.txt (Default)

Seed used

Seed	Origin
1516370207	Randomly generated

Optimised parameters

Prosecution parameters

parameter	estimate	lower bound	upper bound
degradation1	-7.495	-20.000	-1.000
degradation2	-2.785	-20.000	-1.000
degradation3	-2.375	-20.000	-1.000
DNAcont1	21.436	0.000	3416.000
DNAcont2	1091.850	0.000	3416.000
DNAcont3	169.513	0.000	3416.000
scale	67.859	0.000	1000.000
gradientS	0.005	0.000	0.010
gradientAdjust1	0.873	0.200	5.000
gradientAdjust2	1.398	0.200	5.000
gradientAdjust3	0.888	0.200	5.000
gradientAdjust4	1.305	0.200	5.000
gradientAdjust5	0.756	0.200	5.000
gradientAdjust6	1.197	0.200	5.000
gradientAdjust7	1.140	0.200	5.000
gradientAdjust8	1.200	0.200	5.000
gradientAdjust9	0.931	0.200	5.000
gradientAdjust10	0.784	0.200	5.000
gradientAdjust11	1.030	0.200	5.000
gradientAdjust12	0.830	0.200	5.000
gradientAdjust13	0.766	0.200	5.000
gradientAdjust14	1.471	0.200	5.000
gradientAdjust15	0.809	0.200	5.000
gradientAdjust16	1.067	0.200	5.000
repAdjust1	1.125	0.200	5.000
repAdjust2	1.154	0.200	5.000

parameter	estimate	lower bound	upper bound
meanD	0.002	0.000	0.100
meanO	0.002	0.000	0.100
dropin	183.800	5.000	250.000
dropinDeg	-2.428	-20.000	-1.000

Defence parameters

parameter	estimate	lower bound	upper bound
degradation1	-2.261	-20.000	-1.000
degradation2	-10.838	-20.000	-1.000
degradation3	-2.813	-20.000	-1.000
DNAcont1	169.044	0.000	3416.000
DNAcont2	24.849	0.000	3416.000
DNAcont3	1087.574	0.000	3416.000
scale	69.170	0.000	1000.000
gradientS	0.006	0.000	0.010
gradientAdjust1	0.969	0.200	5.000
gradientAdjust2	1.260	0.200	5.000
gradientAdjust3	1.192	0.200	5.000
gradientAdjust4	1.453	0.200	5.000
gradientAdjust5	0.806	0.200	5.000
gradientAdjust6	1.134	0.200	5.000
gradientAdjust7	1.135	0.200	5.000
gradientAdjust8	1.148	0.200	5.000
gradientAdjust9	0.891	0.200	5.000
gradientAdjust10	0.758	0.200	5.000
gradientAdjust11	0.957	0.200	5.000
gradientAdjust12	0.799	0.200	5.000
gradientAdjust13	0.955	0.200	5.000
gradientAdjust14	1.368	0.200	5.000
gradientAdjust15	0.761	0.200	5.000
gradientAdjust16	0.993	0.200	5.000
repAdjust1	1.121	0.200	5.000
repAdjust2	1.150	0.200	5.000
meanD	0.002	0.000	0.100
meanO	0.001	0.000	0.100

parameter	estimate	lower bound	upper bound
dropin	185.049	5.000	250.000
dropinDeg	-2.494	-20.000	-1.000

Runtime

Parameter	Time
elapsed	6.54 hours
start	2018-01-19 13:32:28
end	2018-01-19 20:04:51

System information

Type	Details
Date report generated:	Fri Feb 9 16:34:21 2018
Package	likeLTD
Title	Tools to Evaluate DNA Profile Evidence
Description	Tools to determine DNA profile Weight of Evidence. For further information see the 'likeLTD' guide provided, Balding, D.J. (2013) <DOI:10.1073/pnas.1219739110>, or Steele, C.D. et al. (2016) <DOI:10.1515/sagmb-2016-0038>.
Depends	R (≥ 2.10), DEoptim, ggplot2, gtools, rtf
Suggests	svUnit, scales
Imports	gdata, tools, tcltk
Version	6.3.0
Date	2017-06-11
Author	David Balding, Adrian Timpson, Christopher Steele, Mayeul d'Avezac, James Hetherington.
Maintainer	Christopher Steele <c.steele.11@ucl.ac.uk>
License	GPL-3
URL	https://sites.google.com/site/baldingstatisticalgenetics/
NeedsCompilation	yes
Packaged	2018-02-09 16:17:20 UTC; chris
Built	R 3.3.1; x86_64-pc-linux-gnu; 2018-02-09 16:17:20 UTC; unix
sysname	Linux
release	4.4.0-109-generic

Type	Details
version	#132-Ubuntu SMP Tue Jan 9 19:52:39 UTC 2018
nodename	chris-OptiPlex-9010
machine	x86_64
login	unknown
user	chris
effective_user	chris

C Laboratory protocol

To generate mixtures for validation purposes cheek swab samples were collected from 36 donors. DNA was extracted using a PrepFiler Express BTA™ Forensic DNA Extraction Kit and the Life Technologies Automate Express™ Instrument as per the manufacturer's recommendations.

Single-contributor and multi-contributor samples were created from the 36 DNA samples as shown in Table 7. These created samples were amplified using the AmpFℓSTR® NGMSelect® PCR kit as per the manufacturer's recommendations on a Veriti® 96-Well Fast Thermal Cycler for 30 cycles. The amplified PCR products were size separated by capillary electrophoresis using an ABI 3130 Sequencer, with 1 µL of the PCR products, 10 second injections and 3kV voltage. The results were analysed using GeneMapper® ID v3.2 with a detection threshold of 20 RFU, and no stutter threshold, so that both non-allelic and allelic peaks were recorded.

# Cont	Single replicate			Multiple replicates		
	# Samples	Condition	# Reps	# Samples	Condition	# Reps
1	9	250	x1			
	9	62	x1			
	9	16	x1			
	9	4	x1			
2	12	Maj:Min (250:16)	x1	4	Maj:Min/2	x2
				4	Maj:Min/3	x3
				4	Maj:Min/4	x4
	12	Equal (31:31)	x1	4	Equal/2	x2
				4	Equal/3	x3
				4	Equal/4	x4
3	6	Unequal (250:62:16)	x1	2	Unequal/2	x2
				2	Unequal/3	x3
				2	Unequal/4	x4
	6	Equal (31:31:31)	x1	2	Equal/2	x2
				2	Equal/3	x3
				2	Equal/4	x4

Table 7: Laboratory protocol for generation of single-contributor and multiple-contributor CSPs from 36 donated DNA samples.

Peak height CSPs were converted to discrete CSPs using the same protocol as is used to which which peaks are called as allelic for the allele report (Table 1). Designations defaulted to the lowest confidence of calling a peak if a peak had multiple possible designations e.g. if we have a CSP with peaks 13,14,15 and peak heights 800,35,600, the 14 allele would be called as non-allelic if believed to be an OS of the 13 allele ($x = 0.044$), but uncertain if believed to be a S of the 15 allele ($x = 0.058$). In this situation the allelic call defaults to non-allelic due to the non-allelic call from the 13 parent peak.