A tutorial for the R package *forensim*

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Contents

1 Introduction

This tutorial is a presentation of the forensim package for the R software [1, 2]. forensim is dedicated to the interpretation of forensic DNA mixtures through statistical methods. It also provides simulation tools that allow the generation of genetic data commonly encountered in forensic casework.

In this tutorial, I first introduce forensim object classes. Then, I present statistical tools for forensic DNA mixtures interpretation. Finally, various functionalities of forensim are explored. For all addressed topics, practical and reproducible examples are given.

2 Getting started

2.1 forensim installation

Latest stable version. To be sure to get the latest stable version, download the forensim package (according to your platform) on forensim web page: `http://forensim.r-forge.r-project.org/`. The current version of the package is 1.1-1 and is compatible with R 2.9.1.

The latest developpement build. forensim is hosted by Rforge, the developpment version of the package, resulting from the nightly build, can be obtained by typing the following command lines:
- Under Windows and Linux

```
> install.packages("forensim",repos="http://r-forge.r-project.org")
```

- Under the MacOS system

```
> install.packages("forensim", repos="http://r-forge.r-project.org", type = 'source')
```

Once the package is downloaded to your system, it must be loaded:

```
> library(forensim)

   ### forensim 1.1.1 is loaded ###
```

2.2 How to get help

- The mailing list: please ask questions on forensim mailing list, `forensim-help@lists.r-forge.r-project.org`
- The help pages: classes and functions are documented in the help pages, type ?forensim in R to get an overview of the package.
- The forensim package manual: a compilation of all the help pages in a single pdf file, it can be found at: `http://forensim.r-forge.r-project.org/`

3 Generating data in forensim

forensim provides object classes that facilitate the generation and the storage of data that is commonly encountered in forensic casework: population allele frequencies, individual genotypes and DNA mixtures. Thus, three classes of objects are defined in forensim:

- tabfreq objects: used to store allele frequencies
- simugeno objects: used to store genotypes
- simumix objects: used to store DNA mixtures

forensim objects have the particularity that they can either be used to store pre-existing data, such as allele frequencies in a given population, or simulated data. Creating forensim objects is achieved using specific functions, called constructors, that have the same names than the object they are linked to.

3.1 tabfreq objects

In forensim, allele frequencies are stored in tabfreq objects. Importing data into tabfreq objects is achieved using the tabfreq constructor. The input data must be an object of type data frame[1] or matrix. This object must have the format of the *Journal of Forensic Sciences* for Short Tandem Repeat (STR) loci data: allele names (the number of tandem repeats in case of STR loci) are given in the first column, and frequencies for a given allele are read in rows for different loci given in columns. When an allele is not observed for a given locus, value is coded "NA"[2]. Note that even if the requested input format is based on STR data, different kinds of markers can be imported in forensim.
As an example, we will be using a data set included in forensim:

```
> data(Tu)
```

What is the class of object Tu ?

```
> class(Tu)
```

```
[1] "data.frame"
```

Tu is a data frame giving the allele frequencies for 15 STR loci commonly used in forensic studies, in the Tu Chinese population [3] (see ?Tu). Note that the data set is imported using the command data.
Displaying the first rows (command head):

```
> head(Tu)
```

[1] in R a data frame is a collection of variables, possibly of different types

[2] non observed alleles are coded "-" in the Journal of Forensic Sciences

```
  Allele D8S1179 D21S11 D7S820 CSF1PO D3S1358   TH01 D13S317 D16S539 D2S1338
1    6.0      NA     NA     NA     NA      NA 0.1151      NA      NA      NA
2    7.0      NA     NA 0.0033 0.0034      NA 0.2599      NA      NA      NA
3    8.0  0.0098     NA 0.1382 0.0034      NA 0.0559  0.2712  0.0097      NA
4    9.0      NA     NA 0.0493 0.0582      NA 0.4605  0.1503  0.2305      NA
5    9.2      NA     NA 0.0033     NA      NA     NA      NA      NA      NA
6    9.3      NA     NA     NA     NA      NA 0.0691      NA      NA      NA
  DS19S433 vWA   TPOX D18S51 D5S818 FGA
1       NA  NA     NA     NA     NA  NA
2       NA  NA     NA     NA 0.0097  NA
3       NA  NA 0.5359     NA     NA  NA
4       NA  NA 0.1340     NA 0.0487  NA
5       NA  NA     NA     NA     NA  NA
6       NA  NA     NA     NA     NA  NA
```

This data frame is converted into a tabfreq object by the tabfreq constructor:

```
> tupop <- tabfreq(tab = Tu, pop.names = as.factor("Tu"))
```

The population name is specified as a factor in the pop.names argument.

```
> is.tabfreq(tupop)
```

```
[1] TRUE
```

tupop is a tabfreq object:

```
> tupop
```

```
   # Tabfreq object: allele frequencies  #


@tab: list of allele frequencies
@which.loc: vector of  15  locus names
@pop.names:  populations names
```

As a formal class object, tabfreq is constituted of different 'slots' that contain different types of information. Each slot can be accessed using '@' or the '$' operator that have been implemented for all forensim objects.
Allele frequencies are stored in the @tab slot. For example, frequencies for locus FGA are given by:

```
> tupop$tab$Tu$FGA
```

```
    18     19   19.2     20     21     22   22.2     23   23.2     24     25
0.0392 0.0686 0.0033 0.0458 0.0980 0.1765 0.0033 0.1961 0.0098 0.2222 0.1013
  25.2     26   26.2     27
0.0065 0.0131 0.0065 0.0098
```

Population names are stored in the @pop.names argument:

```
> tupop$pop.names
```

```
[1] Tu
Levels: Tu
```

Finally, locus names appearing in @tab can be accessed elsewhere:

```
> tupop$which.loc
```

```
 [1] "D8S1179"  "D21S11"   "D7S820"   "CSF1PO"   "D3S1358"  "TH01"
 [7] "D13S317"  "D16S539"  "D2S1338"  "DS19S433" "vWA"      "TPOX"
[13] "D18S51"   "D5S818"   "FGA"
```

Note that if several populations are imported in the same tabfreq object, data frames (or matrices) must be given as a list of data frames (or matrices) in the tab argument. In this case, the pop.names argument, which is optional when a single population is handled, becomes obligatory in order to distinguish the populations.

3.2 simugeno objects

simugeno objects are used to store simulated genotypes from a tabfreq object. simugeno objects are created from tabfreq objects by specifying the number of individuals to simulate in the n argument.

At a given locus, an individual's genotype is simulated by randomly drawing two alleles (with replacement) at their respective allele frequencies in the target population.

The loci to take into account for the simulation are given in the which.loc argument. For the illustration purpose, 10 individuals are simulated and only three loci are chosen: D8S1179, TH01 and FGA.

```
> tugeno <- simugeno(tab = tupop, n = 10, which.loc = c("D8S1179",
+     "TH01", "FGA"))

> tugeno

   # Simugeno object: simulated genotypes #


@which.loc: vector of  3  locus names
@nind: 10
@indID:  vector of the individuals ID
@tab.geno:  10 x 3 data frame of genotypes
@tab.freq:  allele frequencies for the 3  loci

Population-related information:
@pop.names: population names
@popind: factor giving the population of each individual
```

@tab.geno is a matrix of 10 genotypes simulated from the allele frequencies of the Tu population. For instance, the genotypes of the five first simulated individuals are:

```
> tugeno$tab.geno[1:5, ]

     D8S1179 TH01     FGA
ind1 "14/10" "9.3/7"  "22/20"
ind2 "14/14" "7/9"    "21/25"
ind3 "15/11" "10/9.3" "22/25"
ind4 "17/13" "9/9"    "25/20"
ind5 "12/13" "7/9.3"  "19/23"
```

The genotype of a homozygous individual carrying the allele 9 is coded ”9/9”. A heterozygous individual carrying alleles 8 and 10 is coded ”8/10”.

Allele frequencies of the population are stored in the slot @tab.freq:

```
> tugeno$tab.freq

$Tu
$Tu$D8S1179
     8     10     11     12     13     14     15     16     17
0.0098 0.0784 0.0784 0.1046 0.2876 0.1863 0.1634 0.0719 0.0196

$Tu$TH01
     6      7      8      9    9.3     10
0.1151 0.2599 0.0559 0.4605 0.0691 0.0395

$Tu$FGA
    18     19   19.2     20     21     22   22.2     23   23.2     24     25
0.0392 0.0686 0.0033 0.0458 0.0980 0.1765 0.0033 0.1961 0.0098 0.2222 0.1013
  25.2     26   26.2     27
0.0065 0.0131 0.0065 0.0098
```

simugeno objects also contain information about the simulated individuals, their (default) ID:

```
> tugeno@indID
```

```
 [1] "ind1"  "ind2"  "ind3"  "ind4"  "ind5"  "ind6"  "ind7"  "ind8"  "ind9"
[10] "ind10"
```

and their population names:

```
> tugeno@popind
```

```
 [1] Tu Tu Tu Tu Tu Tu Tu Tu Tu Tu
Levels: Tu
```

3.3 simumix objects

simumix objects store DNA mixtures. Mixtures can be created from simugeno objects using the constructor simumix. The number of contributors is specified in the argument ncontri.
DNA mixtures are created by randomly drawing individual genotypes with a uniform probability. If there are N individuals in the sample (the simugeno object), then each individual has a probability of $\frac{1}{N}$ to be selected.

```
> mix2 <- simumix(tugeno, ncontri = 2)
```

Constructor simumix has also a which.loc argument, which is by default set to NULL, corresponding to all loci taken into account.

```
> mix2
```

```
   # Simumix object: simulated mixture #


@which.loc: vector of  3  locus names
@ncontri: 2
@mix.prof:  2 x 3 data frame of the contributors genotypes
@mix.all: list of the alleles found in the mixture
@popinfo: populations of the contributors
```

simumix objects keep two types of information: information usually available when dealing with practical cases of forensic DNA mixtures: the alleles present by locus,

```
> mix2$mix.all
```

```
$D8S1179
[1] "12" "13" "14" "15"

$TH01
[1] "6"   "7"   "9"   "9.3"

$FGA
[1] "19" "20" "22" "23"
```

and information that is usually not available: the number of simulated contributors

```
> mix2@ncontri

[1] 2
```

and their genetic profiles:

```
> mix2$mix.prof

     D8S1179 TH01    FGA
ind5 "12/13" "7/9.3" "19/23"
ind9 "15/14" "9/6"   "22/20"
```

3.4 Allele frequencies simulation

In the following, we denote L a locus with k alleles and the ith allele frequency at this locus, in a given population, is denoted p_i.

3.4.1 The homogeneous population case

In forensim, allele frequencies for a single non subdivided population are simulated using the simufreqD function.

Principle

The vector of allele frequencies at locus L is simulated as a vector of random deviates of the Dirichlet distribution [4] with a vector of parameters $(\alpha_1, ..., \alpha_k)$:

$(p_1, ..., p_k) \rightsquigarrow Dirichlet(\alpha_1, ..., \alpha_k)$

An example

5 loci (argument nloc=5) having 2, 3, 4, 5 and 6 alleles respectively (argument na) are simulated:

```
> simufreqD(nloc = 5, na = c(2, 3, 4, 5, 6), alpha = 1)

  Allele Marker1 Marker2 Marker3 Marker4 Marker5
1      1    0.73   0.049   0.280   0.520   0.110
2      2    0.27   0.280   0.029   0.084   0.062
3      3      NA   0.670   0.220   0.230   0.370
4      4      NA      NA   0.470   0.150   0.022
5      5      NA      NA      NA   0.020   0.340
6      6      NA      NA      NA      NA   0.091
```

Argument alpha is the parameter of the Dirichlet distribution. Setting a single value for alpha means that all alleles for all loci are simulated with the same value; this can be changed by giving the appropriate values in alpha, for further details please type '?simufreqD'.
Setting alpha to 1, leads to the generation of allele frequencies as random deviates from a uniform Dirichlet distribution, this means that allele frequencies could take any value varying from 0 to 1, with equal probabilities. Note that the simulated data is in the format of the *Journal of Forensic Sciences* for STR loci data.

3.4.2 The subdivided population case

Principle

The simupopD function simulates subpopulations allele frequencies for independent loci, from a given reference population, following a Dirichlet model.
Allele frequencies in the subpopulations are generated as random deviates from a Dirichlet distribution, the parameters of which control the deviation of allele frequencies from the values in the reference population.
Each allele frequency is modeled as a random variable; with a parameter
$\alpha_i = \frac{p_i(1-\theta)}{\theta}$, where θ is Wright's *Fst* coefficient which allows here accounting for population subdivision [5, 6]. The vector of allele frequencies at a given locus, for a given population, is obtained by:

$$(p_1, ..., p_k) \rightsquigarrow Dirichlet\left(\alpha_1 = \frac{p_1(1-\theta)}{\theta}, ..., \alpha_k = \frac{p_k(1-\theta)}{\theta}\right)$$

An example

In the following example we simulate allele frequencies in two subpopulations: the global population is taken as the Tu Chinese population, and three STR loci are chosen: FGA, TH01 and TPOX. The strength of the deviation from the reference allele frequencies is specified in argument alpha1 for each simulated subpopulation, here we choose 0.01 for the first population and 0.3 for the second one:

```
> simpop1 <- simupopD(npop = 2, globalfreq = Tu, which.loc = c("FGA",
+     "TH01", "TPOX"), alpha1 = c(0.01, 0.3))
```

simpop1 is a list of two tabfreq object; the first one contains allele frequencies used for the simulation (from the Tu population):

```
> simpop1$globfreq

   # Tabfreq object: allele frequencies  #


@tab: list of allele frequencies
@which.loc: vector of  3  locus names
@pop.names:  - empty -
```

the second tabfreq object contains the subpopulations allele frequencies:

```
> simpop1$popfreq

   # Tabfreq object: allele frequencies  #


@tab: list of allele frequencies
@which.loc: vector of  3  locus names
@pop.names:  populations names
```

The simulated subpopulations have the following (default) names:

```
> simpop1$popfreq$pop.names

[1] pop1 pop2
Levels: pop1 pop2
```

4 Statistical methods for forensic DNA mixtures interpretation

Several statistical methods dedicated to the interpretation of forensic DNA mixtures are implemented in forensim:

4.1 The maximum allele count

This method consists in setting the lower bound on the number of contributors to a mixture to the minimum required to explain the observed profiles [7]. For instance, if a mixture shows at three loci, 1, 3 and 4 alleles, then the number of contributors is bounded to 2 $\binom{4}{2}$ contributors.

To exemplify this method, let us simulate a 3-person mixture from the strusa data set, using the allele frequencies from the Caucasian population [8] (see ?strusa):

```
> data(strusa)
> class(strusa)
```

```
[1] "tabfreq"
attr(,"package")
[1] ".GlobalEnv"
```

```
> strusa
```

```
   # Tabfreq object: allele frequencies  #


@tab: list of allele frequencies
@which.loc: vector of  15  locus names
@pop.names:  populations names
```

strusa is a tabfreq object that contains multiple populations:

```
> strusa$pop.names
```

```
[1] Afri Cauc Hisp
Levels: Afri Cauc Hisp
```

thus, the number of genotypes to simulate must be specified in each population (argument n):

```
> geno <- simugeno(tab = strusa, n = c(0, 100, 0))
```

100 genotypes are simulated from the Caucasian population allele frequencies, no genotypes are simulated from the other two populations.
A 3-person mixture is simulated by randomly drawing three contributors from these 100 simulated individuals. The number of contributors in each population must be specified:

```
> mix3 <- simumix(tab = geno, ncontri = c(0, 3, 0))
```

The minimum number of contributors required is computed by the mincontri function. This number can either be computed from all available loci simultaneously (in this default case, the argument loc is set to NULL),

```
> mincontri(mix3, loc = NULL)
```

```
[1] 3
```

or be computed for a specific locus, for example, D8S1179:

```
> mincontri(mix3, loc = "D8S1179")
```

```
[1] 2
```

4.2 The maximum likelihood estimator

The main characteristic of this method is that it takes into account allele frequencies in the estimations. The likelihood function is derived from the formula of Curran *et al* [9] for DNA mixtures interpretation, in the particular case where all contributors to the mixture are unknown and there are no typed individuals [10].

4.2.1 Likelihood of the observed alleles at a given locus, conditional on the number of contributors to the mixture

The function lik.loc computes the likelihood of the observed alleles at a given locus, conditional on the number of contributors to the mixture [10]. This function takes in argument the number of contributors x, the mixture as a simumix object, and the allele frequencies given in a tabfreq object. For the previously simulated 3-person mixture mix3,

```
> mix3
```

```
   # Simumix object: simulated mixture #

@which.loc: vector of  15  locus names
@ncontri: 3
@mix.prof:  3 x 15 data frame of the contributors genotypes
@mix.all: list of the alleles found in the mixture
@popinfo: populations of the contributors
```

the likelihood per locus of observing alleles given that 1 individual contributed to the mixture is:

```
> lik.loc(x = 1, mix = mix3, freq = strusa, refpop = "Cauc")
```

```
   CSF1PO       FGA      TH01      TPOX       VWA   D3S1358    D5S818    D7S820
0.0000000 0.0000000 0.0000000 0.0000000 0.0000000 0.0000000 0.0000000 0.0000000
  D8S1179   D13S317   D16S539    D18S51    D21S11   D2S1338   D19S433
0.0000000 0.0000000 0.0000000 0.0000000 0.0000000 0.0000000 0.1124761
```

the likelihood that 3 individuals contributed to the mixture is:

```
> lik.loc(x = 3, mix = mix3, freq = strusa, refpop = "Cauc")
```

```
      CSF1PO          FGA         TH01         TPOX          VWA      D3S1358 
0.3238078841 0.0143969301 0.1495254898 0.2432364946 0.0254440484 0.0053984676 
      D5S818       D7S820      D8S1179      D13S317      D16S539        D18S51 
0.0881518372 0.0215292215 0.0417412582 0.0793305226 0.0618112692 0.0096802390 
      D21S11      D2S1338      D19S433 
0.0053859847 0.0001170379 0.0175766927 
```

Note here that strusa contains three populations, so the reference population, here Caucasians, must be specified in the refpop argument.
The overall likelihood, for all loci characterized in the mixture can be computed using the function lik:

```
> lik(x = 3, mix = mix3, freq = strusa, refpop = "Cauc")
```

```
[1] 9.703005e-25
```

4.2.2 Maximum likelihood estimators

likestim.loc looks for the number of contributors that maximizes the likelihood at each given locus. For the estimations to be biologically plausible, the estimations are restricted to the discrete interval [1,6] [10]. These functions give the number of contributors that maximizes the likelihood (max) and the corresponding likelihood value (maxval). The per locus estimations are:

```
> likestim.loc(mix = mix3, freq = strusa, refpop = "Cauc")
```

```
        max  maxval
CSF1PO    3 0.32000
FGA       3 0.01400
TH01      5 0.22000
TPOX      4 0.26000
VWA       3 0.02500
D3S1358   3 0.00540
D5S818    6 0.19000
D7S820    2 0.04100
D8S1179   3 0.04200
D13S317   4 0.09300
D16S539   4 0.07900
D18S51    4 0.01200
D21S11    4 0.00880
D2S1338   4 0.00021
D19S433   1 0.11000
```

and the estimation using all loci simultaneously is:

```
> likestim(mix = mix3, freq = strusa, refpop = "Cauc")
```

```
     max  maxval
[1,]   3 9.7e-25
```

4.3 The exclusion probability

The exclusion probability, also known as the Random Man Not Excluded (RMNE) is implemented in forensim in the function PE.
The PE function takes a simumix object for which to compute the exclusion probability and the allele frequencies given in a tabfreq object. If the latter contains several populations, than the reference population must be specified in the refpop argument. Implementation of the PE function includes the possibility of correcting for deviation from Hardy Weinberg proportions in the population, due to subdivision, using Wright's *Fst* called here theta [11]:

```
> PE(mix3, strusa, refpop = "Cauc", theta = 0, byloc = TRUE)
```

```
     CSF1PO    FGA   TH01   TPOX   VWA D3S1358 D5S818 D7S820 D8S1179 D13S317
PE_l 0.2271 0.6268 0.1828 0.1947 0.537  0.5392 0.1219 0.6868  0.4548  0.3202
     D16S539 D18S51 D21S11 D2S1338 D19S433
PE_l  0.2786 0.5614 0.4474  0.7614   0.728
```

The row PE_l stands for the exclusion probability per locus, read in column. The byloc argument is a logical indicating whether the exclusion probability should be computed per locus (byloc=TRUE) or for all loci (byloc=FALSE):

```
> PE(mix = mix3, freq = strusa, refpop = "Cauc", theta = 0, byloc = FALSE)
```

```
      PE
0.999953
```

4.4 The random match probability

The Random Match Probability (RMP) is computed using the RMP function which implements the formulas gave by Balding and Nichols [12]. The suspect's profile can either be given directly in R as matrix, or be read from a text file.

DNA evidence as a matrix

```
> data <- matrix(c("CSF1PO", "FGA", "TH01", "TPOX", "VWA", "D3S1358",
+     "D5S818", "D7S820", "D8S1179", "D13S317", "D16S539", "D18S51",
+     "D21S11", "D2S1338", "D19S433", "12/11", "22/19", "6/7",
+     "10/8", "17/18", "18/17", "12/12", "8/8", "13/13", "11/11",
+     "12/10", "14/15", "33.2/32.2", "23/22", "14/14"), nc = 2)
> colnames(data) <- c("locus", "genotype")
> data
```

```
      locus     genotype
 [1,] "CSF1PO"  "12/11"
 [2,] "FGA"     "22/19"
 [3,] "TH01"    "6/7"
 [4,] "TPOX"    "10/8"
 [5,] "VWA"     "17/18"
 [6,] "D3S1358" "18/17"
 [7,] "D5S818"  "12/12"
 [8,] "D7S820"  "8/8"
 [9,] "D8S1179" "13/13"
[10,] "D13S317" "11/11"
[11,] "D16S539" "12/10"
[12,] "D18S51"  "14/15"
[13,] "D21S11"  "33.2/32.2"
[14,] "D2S1338" "23/22"
[15,] "D19S433" "14/14"
```

The random match probability in the unrelated case (unknown offender and suspect are not related) and in absence of population subdivision (theta=0,default case) is given by [1]:

```
> RMP(suspect = data, freq = strusa, refpop = "Cauc")


NOTE: THIS PACKAGE IS NOW OBSOLETE.

  The R-Genetics project has developed an set of enhanced genetics
  packages to replace 'genetics'. Please visit the project homepage
  at http://rgenetics.org for informtion.

$RMP.loc
 CSF1PO     FGA    TH01    TPOX     VWA D3S1358  D5S818  D7S820 D8S1179 D13S317
 0.2200  0.0230  0.0880  0.0600  0.1100  0.0660  0.1500  0.0230  0.0930  0.1200
D16S539  D18S51  D21S11 D2S1338 D19S433
 0.0370  0.0440  0.0045  0.0090  0.1400

$RMP
[1] 6.2e-20
```

In the absence of population subdivision, and in the case where the suspect and an unknown offender are for example siblings, the k argument must be modified from k=(1,0,0) to k=c(1/4,1/2,1/4):

```
> RMP(suspect = data, freq = strusa, k = c(1/4, 1/2, 1/4), refpop = "Cauc")


$RMP.loc
 CSF1PO     FGA    TH01    TPOX     VWA D3S1358  D5S818  D7S820 D8S1179 D13S317
   0.47    0.32    0.38    0.41    0.40    0.36    0.48    0.33    0.43    0.45
D16S539  D18S51  D21S11 D2S1338 D19S433
   0.35    0.34    0.28    0.29    0.47

$RMP
[1] 4.6e-07
```

DNA evidence read from an existing text file The same data is available in a preexisting file “exprofile.txt” from the forensim package, accessed by the system.file command:

```
> RMP(filename = system.file("files/exprofile.txt", package = "forensim"),
+     freq = strusa, refpop = "Cauc")


$RMP.loc
 CSF1PO     FGA    TH01    TPOX     VWA D3S1358  D5S818  D7S820 D8S1179 D13S317
 0.2200  0.0230  0.0880  0.0600  0.1100  0.0660  0.1500  0.0230  0.0930  0.1200
D16S539  D18S51  D21S11 D2S1338 D19S433
 0.0370  0.0440  0.0045  0.0090  0.1400

$RMP
[1] 6.2e-20
```

4.5 Likelihood ratios

Likelihood ratios are computed using the LR function which implements the general formula of Curran *et al* for forensic DNA mixtures interpretation [13].

Locus	Mixture	Victim	Suspect	Frequency
D3S1358	14		14	0.033
	15	15		0.331
	17		17	0.239
	18	18		0.056

Table 1: Alleles from a DNA stain from a rape case in Hong Kong

An example Consider the following genetic profiles from a rape case in Hong Kong [14]:

Locus D3S1358 shows 4 distinct alleles (14, 15, 17 and 18), thus, the number of contributors to the mixed sample is taken 2.

Scenario 1 The following hypotheses are tested:
Prosecution hypotheses Hp: Contributors were the victim and the suspect.
Defense hypotheses Hd: Contributors were 2 unknown people.
First, the genotypes are assigned to the victim and the suspect:

```
> victim <- "15/18"
> suspect <- "14/17"
```

Then, the likelihood ratio is computed using the LR function:

```
> LR(stain = c(14, 15, 17, 18), freq = c(0.033, 0.331, 0.239, 0.056),
+     xp = 0, Tp = c(victim, suspect), Vp = NULL, Td = victim,
+     Vd = NULL, xd = 2)


[1] 37.96
```

The mixture profile is nearly 38 times more likely if it came from the suspect and the victim than if it came from two unknown unrelated individuals from the population of Hong Kong.

Scenario 2 The following hypotheses are tested:
Prosecution hypotheses Hp: Contributors were the victim and the suspect.
Defense hypotheses Hd: Contributors were the victim and one unknown.

```
> LR(stain = c(14, 15, 17, 18), freq = c(0.033, 0.331, 0.239, 0.056),
+     xp = 0, Tp = c(victim, suspect), Vp = NULL, Td = victim,
+     Vd = suspect, xd = 1)


[1] 63.4
```

The mixture profile is 63 times more likely if it came from the suspect than if it came from an unrelated individual from the population of Hong Kong.

[1] RMP calls many functions from the genetics package, which is now obsolete. So, don't worry if you get a warning message from the genetics package.

5 Two-person DNA mixtures resolution using allele peak heights or areas information: The *mastermix* interface

mastermix is a Tcl/Tk graphical user interface dedicated to the resolution of two-person DNA mixtures using allele peak heights or areas information. mastermix is the implementation of a method developed by Gill *et al* [15] and previously programmed into an Excel macro by Dr. Peter Gill.

This method searches through simulation the most likely combination(s) of the contributors' genotypes. Having previously obtained an estimation for the mixture proportion, it is possible to reduce the number of possible genotype combinations by keeping only those supported by the observed data. This is achieved by computing the sum of square differences between the expected allelic ratio and the observed allelic ratio, for all possible mixture combinations. The likelihood of peak heights (or areas), given the combination of genotypes, is high if the residuals are low. Genotype combinations are thus selected according to the peak heights with the highest likelihoods. Appendix A gives the formulas for the expected allelic ratios following from [15].

Typing mastermix() in the R console launches a dialog window (Figure 1):

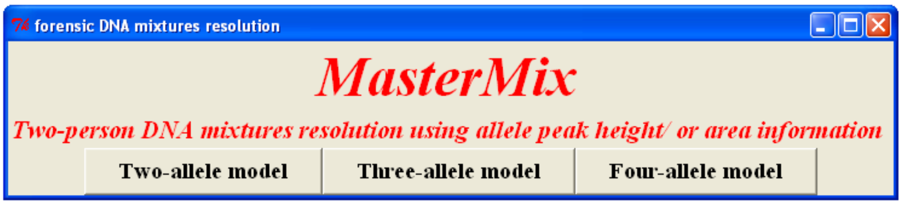
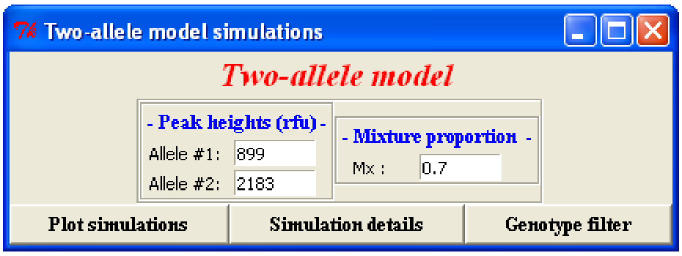

forensic DNA mixtures resolution
MasterMix
Two-person DNA mixtures resolution using allele peak height/ or area information
Two-allele model
Three-allele model
Four-allele model

Figure 1: The mastermix interface

mastermix offers a graphical representation of the simulation for three models:

- The two allele model: at a given locus, two alleles are observed in the DNA stain
- The three allele model: at a given locus, three alleles are observed in the DNA stain
- The four allele model: at a given locus, four alleles are observed in the DNA stain

A left-click on each button launches a simulation dialog window for the corresponding model, while a right-click opens the corresponding help page. For instance, a left-click on the "Two-allele model" button yields Figure 2:



Allele #1: 899

Allele #2: 2183

Mx : 0.7

Figure 2: Two-allele model interface.

Note that default values for peak heights and observed mixture proportion are only given for illustration purposes.
As an example, we suppose that a locus showing four distinct alleles gives an estimation for the mixture proportion of 0.70, and that another locus shows two distinct alleles with heights of 899 and 2183 rfus. A left-click on the “Plot simulations” button yields a graphical representation of the residuals of each possible genotype combinations of the peak areas, for varying values of the mixture proportion across the interval [0.1, 0.9].

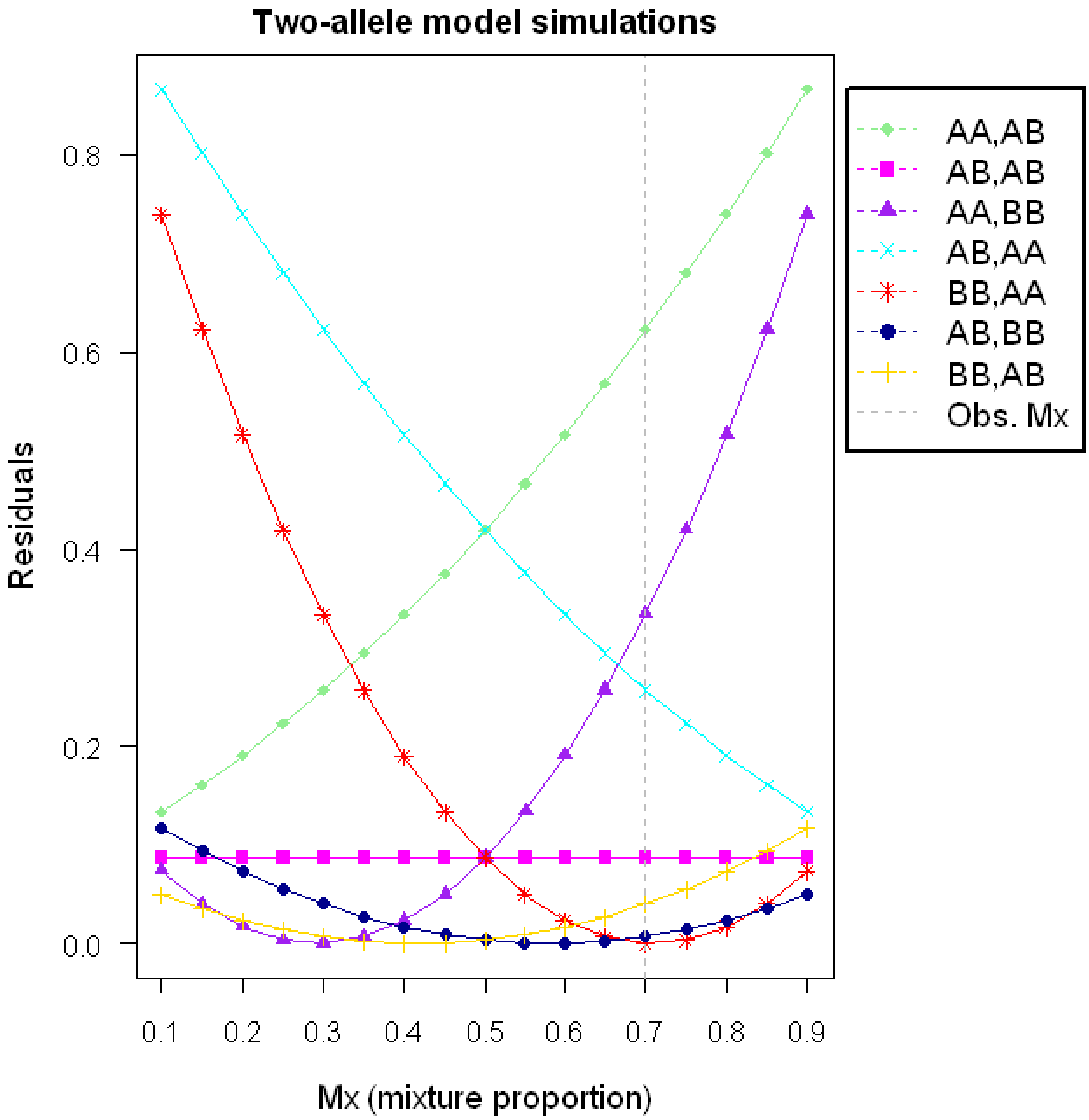
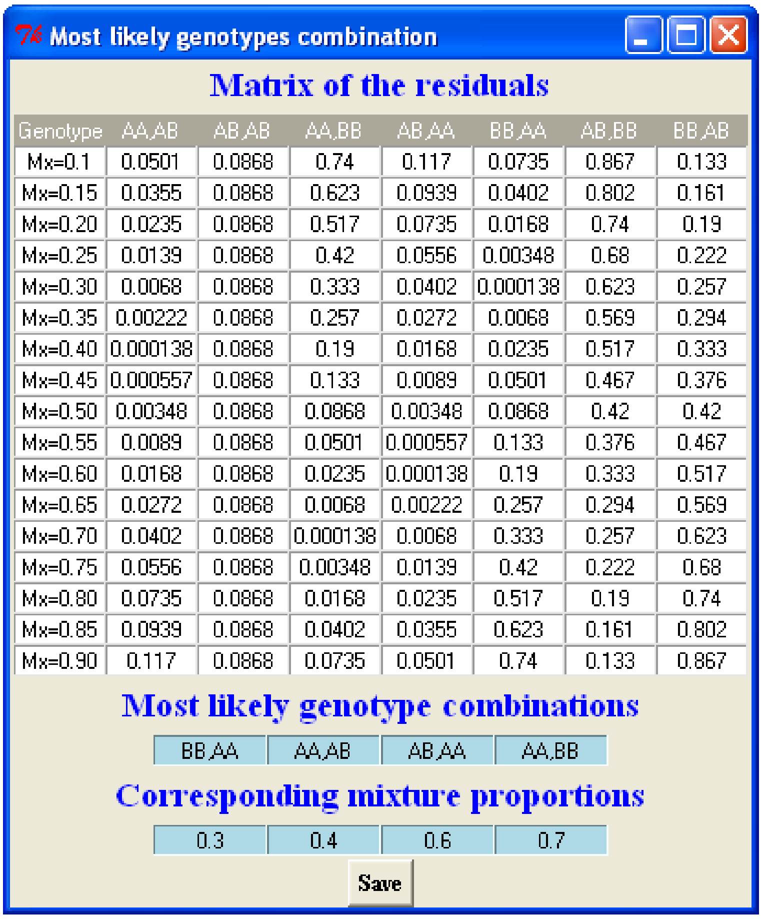


Figure 3: Graphical simulations of the residuals for each possible genotype combination, in a two-allele model, for every possible mixture combination based on variation of the mixture proportion.

The graphical simulation shows that multiple combinations correspond to the lowest residual value. The corresponding numerical results are obtained by clicking the “Simulations details” button:



Matrix of the residuals

Genotype	AA,AB	AB,AB	AA,BB	AB,AA	BB,AA	AB,BB	BB,AB
Mx=0.1	0.0501	0.0868	0.74	0.117	0.0735	0.867	0.133
Mx=0.15	0.0355	0.0868	0.623	0.0939	0.0402	0.802	0.161
Mx=0.20	0.0235	0.0868	0.517	0.0735	0.0168	0.74	0.19
Mx=0.25	0.0139	0.0868	0.42	0.0556	0.00348	0.68	0.222
Mx=0.30	0.0068	0.0868	0.333	0.0402	0.000138	0.623	0.257
Mx=0.35	0.00222	0.0868	0.257	0.0272	0.0068	0.569	0.294
Mx=0.40	0.000138	0.0868	0.19	0.0168	0.0235	0.517	0.333
Mx=0.45	0.000557	0.0868	0.133	0.0089	0.0501	0.467	0.376
Mx=0.50	0.00348	0.0868	0.0868	0.00348	0.0868	0.42	0.42
Mx=0.55	0.0089	0.0868	0.0501	0.000557	0.133	0.376	0.467
Mx=0.60	0.0168	0.0868	0.0235	0.000138	0.19	0.333	0.517
Mx=0.65	0.0272	0.0868	0.0068	0.00222	0.257	0.294	0.569
Mx=0.70	0.0402	0.0868	0.000138	0.0068	0.333	0.257	0.623
Mx=0.75	0.0556	0.0868	0.00348	0.0139	0.42	0.222	0.68
Mx=0.80	0.0735	0.0868	0.0168	0.0235	0.517	0.19	0.74
Mx=0.85	0.0939	0.0868	0.0402	0.0355	0.623	0.161	0.802
Mx=0.90	0.117	0.0868	0.0735	0.0501	0.74	0.133	0.867

Most likely genotype combinations

BB,AA	AA,AB	AB,AA	AA,BB

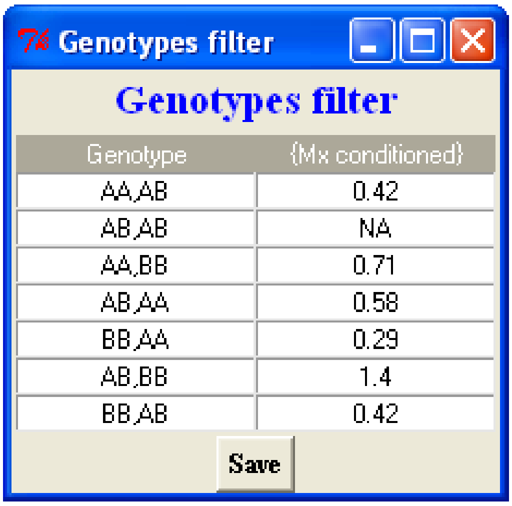
Corresponding mixture proportions

0.3	0.4	0.6	0.7

Figure 4: Numerical results of the graphical simulation.

Genotype combinations having the lowest residuals are highlighted along with the corresponding mixture proportion. The most likely combinations are: (BB,AA), (AA, AB), (AB, AA), (AA, BB) with the corresponding mixtures proportions :0.3, 0.4, 0.5 and 0.7. Note that clinking the "Save" button launches a window where the desired path for the save file can be specified, default creates a text file in the current folder.

The third button, "Genotypes filter" launches a window showing a matrix of the mixture proportion conditional on the genotype combination.



Genotype	{Mx conditioned}
AA,AB	0.42
AB,AB	NA
AA,BB	0.71
AB,AA	0.58
BB,AA	0.29
AB,BB	1.4
BB,AB	0.42

Figure 5: Genotypes filter: Mixture proportion conditional on the genotypes combination.

The mixture proportions conditional on the genotype combination gives a supplementary indication for the reduction of the number of possible combinations: Genotypes with non plausible mixture proportions ranges are not kept. The results confirm that genotypes which have not been already selected during the graphical simulation step, are not supported by the data. Formulas used for the calculations are given in Appendix A.

6 Miscellaneous

6.1 Manipulating forensim objects

forensim objects are mainly formed by lists and data frames. Modification of the slots of an object can easily be done using operators '$' (lists) or '[' (data frame and matrix). For example, we wish to modify the frequencies of a given locus, say FGA, in the tabfreq object tupop:

```
> tupop$tab$Tu$FGA
```

```
    18     19   19.2     20     21     22   22.2     23   23.2     24     25 
0.0392 0.0686 0.0033 0.0458 0.0980 0.1765 0.0033 0.1961 0.0098 0.2222 0.1013 
  25.2     26   26.2     27 
0.0065 0.0131 0.0065 0.0098 
```

Frequencies of alleles 18 and 27 are modified from 0.0392 and 0.0098 to 0.01 and 0.03 respectively:

```
> tupop$tab$Tu$FGA[c("18", "27")] <- c(0.01, 0.03)
> tupop$tab$Tu$FGA
```

```
    18     19   19.2     20     21     22   22.2     23   23.2     24     25 
0.0100 0.0686 0.0033 0.0458 0.0980 0.1765 0.0033 0.1961 0.0098 0.2222 0.1013 
  25.2     26   26.2     27 
0.0065 0.0131 0.0065 0.0300 
```

6.2 How to change population names

Changing population names in any forensim object is achieved using the function changepop. For example, changing the population name in the tabfreq object tupop from “Tu” (argument oldpop) to “Tu2” (argument newpop) is achieved by:

```
> tupop2 <- changepop(tupop, oldpop = "Tu", newpop = "Tu2")
> tupop2@pop.names
```

```
[1] Tu2
Levels: Tu2
```

6.3 How to find the allele frequencies of a mixture

The allele frequencies of a mixture; stored in a simumix object, can be found using the function findfreq. The tabfreq object from which to extract the allele frequencies must be specified. For instance, allele frequencies in object mix3 are found from the Caucasian population:

```
> temp <- findfreq(mix3, freq = strusa, refpop = "Cauc")
> temp
```

```
$Cauc
$Cauc$CSF1PO
     10      11      12
0.21689 0.30132 0.36093

$Cauc$FGA
     21      22      24      25
0.18543 0.21854 0.13576 0.07119

$Cauc$TH01
      6       7       9     9.3
0.23179 0.19040 0.11424 0.36755

$Cauc$TPOX
      8       9      11
0.53477 0.11921 0.24338

$Cauc$VWA
     14      16      17      19
0.09437 0.20033 0.28146 0.10430

$Cauc$D3S1358
     15      16      18      19
0.26159 0.25331 0.15232 0.01159

$Cauc$D5S818
     10      11      12      13
0.05132 0.36093 0.38411 0.14073

$Cauc$D7S820
      8      10      12
0.15066 0.24338 0.16556

$Cauc$D8S1179
     11      12      13      14
0.08278 0.18543 0.30464 0.16556

$Cauc$D13S317
      8      11      12      13
0.11258 0.33940 0.24834 0.12417
```

```
$Cauc$D16S539
     10      11      12      13
0.05629 0.32119 0.32616 0.14570

$Cauc$D18S51
     12      13      14      16      17
0.12748 0.13245 0.13742 0.13907 0.12583

$Cauc$D21S11
     28      29      30    30.2      31
0.15894 0.19536 0.27815 0.02815 0.08278

$Cauc$D2S1338
     18      19      21      22      24      25
0.07947 0.11424 0.04139 0.03808 0.12252 0.09272

$Cauc$D19S433
     14      15
0.36921 0.15232
```

temp is a list of a single element ”Cauc”, which contains also a list:

```
> class(temp$Cauc)
```

```
[1] "list"
```

Allele frequencies of locus TPOX for example, are given by:

```
> temp$Cauc$TPOX
```

```
      8       9      11
0.53477 0.11921 0.24338
```

6.4 The number of alleles in a mixture

The number of alleles in a simumix object can be determined by the function nball. The overall loci number of alleles in the 2-person mixture mix2 is:

```
> nball(mix2, byloc = FALSE)
```

```
[1] 12
```

and the numbers of alleles per locus can be obtained by setting the argument byloc to TRUE:

```
> nball(mix2, byloc = TRUE)
```

```
D8S1179    THO1     FGA
      4       4       4
```

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A Appendix: Formulas used in *mastermix*

A.1 Expected allelic ratios

Two-allele model: expected allelic ratios conditional on each possible genotype combination of the contributors to the mixture, when two alleles, A and B (in ascending order of molecular weights) are observed at a given locus, and $\hat{M}_x$ is the proportion of sample from the first contributor [15].

Combination	Alleles	
	A	B
AA,AB	$\frac{\hat{M}_x}{2} + 0.5$	$\frac{1 - \hat{M}_x}{2}$
AB,AB	0.5	0.5
AA,BB	$\hat{M}_x$	$1 - \hat{M}_x$
AB,AA	$1 - \frac{\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$
BB,AA	$1 - \hat{M}_x$	$\hat{M}_x$
AB,BB	$\frac{\hat{M}_x}{2}$	$1 - \frac{\hat{M}_x}{2}$
BB,AB	$\frac{1 - \hat{M}_x}{2}$	$\frac{\hat{M}_x}{2} + 0.5$

Three-allele model: expected allelic ratios conditional on each possible genotype combination of the contributors to the mixture when three alleles, A, B and C (in ascending order of molecular weights) are observed at a given locus [15].

Combination	Alleles		
	A	B	C
AA,BC	$\hat{M}_x$	$\frac{1-\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$
BB,AC	$\frac{1-\hat{M}_x}{2}$	$\hat{M}_x$	$\frac{1-\hat{M}_x}{2}$
CC,AB	$\frac{1-\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	$\hat{M}_x$
AB,AC	0.5	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$
BC,AC	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	0.5
AB,BC	$\frac{\hat{M}_x}{2}$	0.5	$\frac{1-\hat{M}_x}{2}$
BC,AA	$1-\hat{M}_x$	$\frac{\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$
AC,BB	$\frac{\hat{M}_x}{2}$	$1-\hat{M}_x$	$\frac{\hat{M}_x}{2}$
AB,CC	$\frac{\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	$1-\hat{M}_x$
AC,AB	0.5	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$
AC,BC	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	0.5
BC,AB	$\frac{1-\hat{M}_x}{2}$	0.5	$\frac{\hat{M}_x}{2}$

Four-allele model: expected allelic ratios conditional on each possible genotype combination of the contributors to the mixture when four alleles, A, B, C and D (in ascending order of molecular weights) are observed at a given locus [15].

Combination	Alleles			
	A	B	C	D
AB,CD	$\frac{\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$
AC,BD	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$
AD,BC	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$
BC,AD	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$
BD,AC	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$
CD,AB	$\frac{1-\hat{M}_x}{2}$	$\frac{1-\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$	$\frac{\hat{M}_x}{2}$

A.2 Conditional mixtures proportions

The following tables give the formulas for the mixture proportion conditional on the genotype combinations. The conditional mixture proportions are computed using observed allele peak heights (or equivalently peak areas) [16].

Mixture proportions conditioned on the genotype combination for a locus showing two alleles, A and B (in ascending order of molecular weights), with peak heights ϕ_A and ϕ_B.

Two-allele model

Genotype combination	Conditional mixture proportion
AA,AB	$\frac{\phi_A - \phi_B}{\phi_A + \phi_B}$
AB,AB	No information is present
AA,BB	$\frac{\phi_A}{\phi_A + \phi_B}$
AB,AA	$\frac{2\phi_B}{\phi_A + \phi_B}$
BB,AA	$\frac{\phi_B}{\phi_A + \phi_B}$
AB,BB	$\frac{2\phi_A}{\phi_A + \phi_B}$
BB,AB	$\frac{\phi_B - \phi_A}{\phi_A + \phi_B}$

Mixture proportions conditioned on the genotype combination for a locus showing three alleles,, A , B and C (in ascending order of molecular weights), with peak heights ϕ_A, ϕ_B and ϕ_C.

Three-allele model

Genotype combination	Conditional mixture proportion
AA,BC	$\frac{\phi_A}{\phi_A + \phi_B + \phi_C}$
BB,AC	$\frac{\phi_B}{\phi_A + \phi_B + \phi_C}$
CC,AB	$\frac{\phi_C}{\phi_A + \phi_B + \phi_C}$
AB,AC	$\frac{\phi_B}{\phi_B + \phi_C}$
BC,AC	$\frac{\phi_B}{\phi_A + \phi_B}$
AB,BC	$\frac{\phi_A}{\phi_A + \phi_C}$
BC,AA	$\frac{\phi_B + \phi_C}{\phi_A + \phi_B + \phi_C}$
AC,BB	$\frac{\phi_A + \phi_C}{\phi_A + \phi_B + \phi_C}$
AB,CC	$\frac{\phi_A + \phi_B}{\phi_A + \phi_B + \phi_C}$
AC,AB	$\frac{\phi_C}{\phi_B + \phi_C}$
AC,BC	$\frac{\phi_A}{\phi_A + \phi_B}$
BC,AB	$\frac{\phi_C}{\phi_A + \phi_C}$

Mixture proportions conditioned on the genotype combination for a locus showing four alleles, A , B, C and D (in ascending order of molecular weights), with peak heights ϕ_A, ϕ_B, ϕ_C and ϕ_D.

Four-allele model

Genotype combination	Conditional mixture proportion
AB,CD	$\frac{\phi_A + \phi_B}{\phi_A + \phi_B + \phi_C + \phi_D}$
AC,BD	$\frac{\phi_A + \phi_C}{\phi_A + \phi_B + \phi_C + \phi_D}$
AD,BC	$\frac{\phi_A + \phi_D}{\phi_A + \phi_B + \phi_C + \phi_D}$
BC,AD	$\frac{\phi_B + \phi_C}{\phi_A + \phi_B + \phi_C + \phi_D}$
BD,AC	$\frac{\phi_B + \phi_D}{\phi_A + \phi_B + \phi_C + \phi_D}$
CD,AB	$\frac{\phi_C + \phi_D}{\phi_A + \phi_B + \phi_C + \phi_D}$