Manual for EuroForMix v1

Author: Øyvind Bleka <Oyvind.Bleka.at.fhi.no>
Date: 01-06-2015

(A) Installation and running program:

1) Run R (>=3.0.1) in Windows, Linux or MAC (http://cran.r-project.org/).
2) Required packages to run GUI:
    a. gWidgetstcltk (depends on digest,tcltk)
    b. gWidgets
3) Other required packages:
    a. cubature
        i. Required for multivariate integration (Integrated LR).
    b. forensim
        i. Required for qualitative Weight-of-Evidence.
4) Installation and run gammadnamix:
    a. install.packages("gammadnamix", repos="http://R-Forge.R-project.org")
    b. library(gammadnamix)
    c. euroformix()

(B) GUI

Sections:

0- Toolbar
1- Importing data
2- Model specification
3- MLE fit: (‘Continuous LR (Maximum Likelihood based)’)
4- Deconvolution (Deconvolution based on the continuous model)
5- Database Search (Database search based on the continuous and qualitative model)
6- Qual.LR (Qualitative model)
7- Generate data (Generation from the continuous model)

0. Toolbar

- File

  - **Set directory**: The user may select the working directory of the R-program.

  - **Open project**: The user may open an earlier project which is saved in a file in the form: "projectname.Rdata".

  - **Save project**: The user may save the existing project into a file with name: "projectname".
    - Extension .Rdata is added automatically to project name.
    - All data imported to the program and resulting calculations are stored into a single project-file which may be opened at any time in the program.
    - Saving a project has the following advantages:
      - Large reference databases are stored efficiently (the required space for the database is drastically reduced).

  - **Quit project**: When button is pushed, the user is given a question about saving project before terminating the GUI.

- Frequencies

  - **Set size of frequency database**: User may specify number of samples 'N' used to create the population frequencies.
    - When new alleles, i.e. not in the frequency database, from imported files are found, these are assigned as freq0.
      - If N=0 (this is default), freq0 is equal to the minimum imported allele frequency.
      - If N>0, freq0='5/(2N)'.
    - New alleles are updated to the population frequency database:
      - When a reference database is imported.
      - When interpretations are carried out ('Generate sample', Deconvolution, Weight-of-Evidence or 'Database search')
      - Frequencies are normalized for each of these two cases:
        - **WARNING**: Normalizing (requiring sum of frequencies equal 1) of the assumed allele frequencies are carried out twice if:
          - New alleles (not observed in the allele frequency file) are observed in the imported reference database and again other new alleles are observed in the imported evidence/reference profiles.

  - **Set number of wildcards in false positive match**: The user may specify the number of 'wildcards' in the random match probability statistics, which are applied when the user has imported and selected an evidence stain together with the population frequencies.

- Optimization

    - **Set number of random startpoints**: The user may set required number of independent random startpoints in the optimizer to ensure that the global maximum is attained for the Maximum Likelihood Estimator (MLE). Default is 3.

    - **Set variance of randomizer**: The user may set the variance parameter used for the random generation of startpoints used in optimizer. Default is 10**.**

- MCMC (Markov Chain Monte Carlo)

    - **Set number of samples**: The user may set the number of samples drawn from the posterior distribution of the parameters. Default is 10000.

    - **Set variance of randomizer**: The user may set the variance parameter scalar used in the 'Markov Chain Monte Carlo (MCMC) random walk Metropolis'. See vignette for details. Default is 10.
        - Note that this value should be tweaked so that the acceptance rate of the sampler is around 0.2 (to ensure global exploration in the parameter space).

- Integration

    - **Set relative error requirement**: The user may set the required estimated relative error used in the integration function adaptIntegrate {cubature}. See vignette for details. Default is 0.005.

    - **Set maximum of mu-parameter**: The user may set upper limit of mu-parameter (mean peak height). See vignette for details. Default is 21000.

    - **Set maximum of sigma-parameter**: The user may set upper limit of sigma-parameter (coefficient of variation of peak heights). See vignette for details. Default is 1.

    - **Set maximum of stutter rate-parameter**: The user may set upper limit of the (n-1)-stutter rate parameter (xi). More details about the stutter rate is given under 'Advanced Parameters' in the Model specification section. Default is 1.

- Deconvolution

    - **Set required summed probability**: The user may set the required summed posterior genotype-probability which the deconvoluted list must contain. Default is 0.9999.

    - **Set max listsize:** The user may set maximum number of elements in the deconvoluted list. Default is 20.

        - The greater max listsize, the more time-consuming (and memory consuming) the search-algorithm behind will be.

- Database search

    - **Set maximum view-elements**: The user may set maximum number of individuals to show from the reference-database. Default is 10000.
        - The greater this 'value', the more time-consuming it will become to show the table on the screen.
        - Note that the results table from the database search shows only the top 'value'-ranked elements.

    - **Set drop-in probability for qualitative model**: When searching database with continuous LR model, the qualitative LR model is also considered with a specific drop-in probability parameter given here (default is 0.05).

- Qual LR

    - **Set upper range for sensitivity**: The user may specify the maximum allele dropout-probability in the sensitivity plot (for a qualitative model). Default is 0.6.

    - **Set nticks for sensitivity**: The user may specify number of grids of the allele dropout-probability in the sensitivity plot (for a qualitative model). Default is 32.

    - **Set required samples in dropout distr.**: The user may specify number of required allele drop-out probability samples used to estimate the quantiles or median for the distribution of the '*allele drop-out probability given number of observed alleles*'.

    - **Set significance level in dropout distr.**: The user may specify the significance level in the conservative LR calculation (i.e. the quantile for the distribution of the '*allele drop-out probability given number of observed alleles*'). Default is 0.05.

    - **Set number of non-contributors**: The user may specify number of random non-contributor samples in the non-contributor analysis. Default is 1e6.

1. Importing data

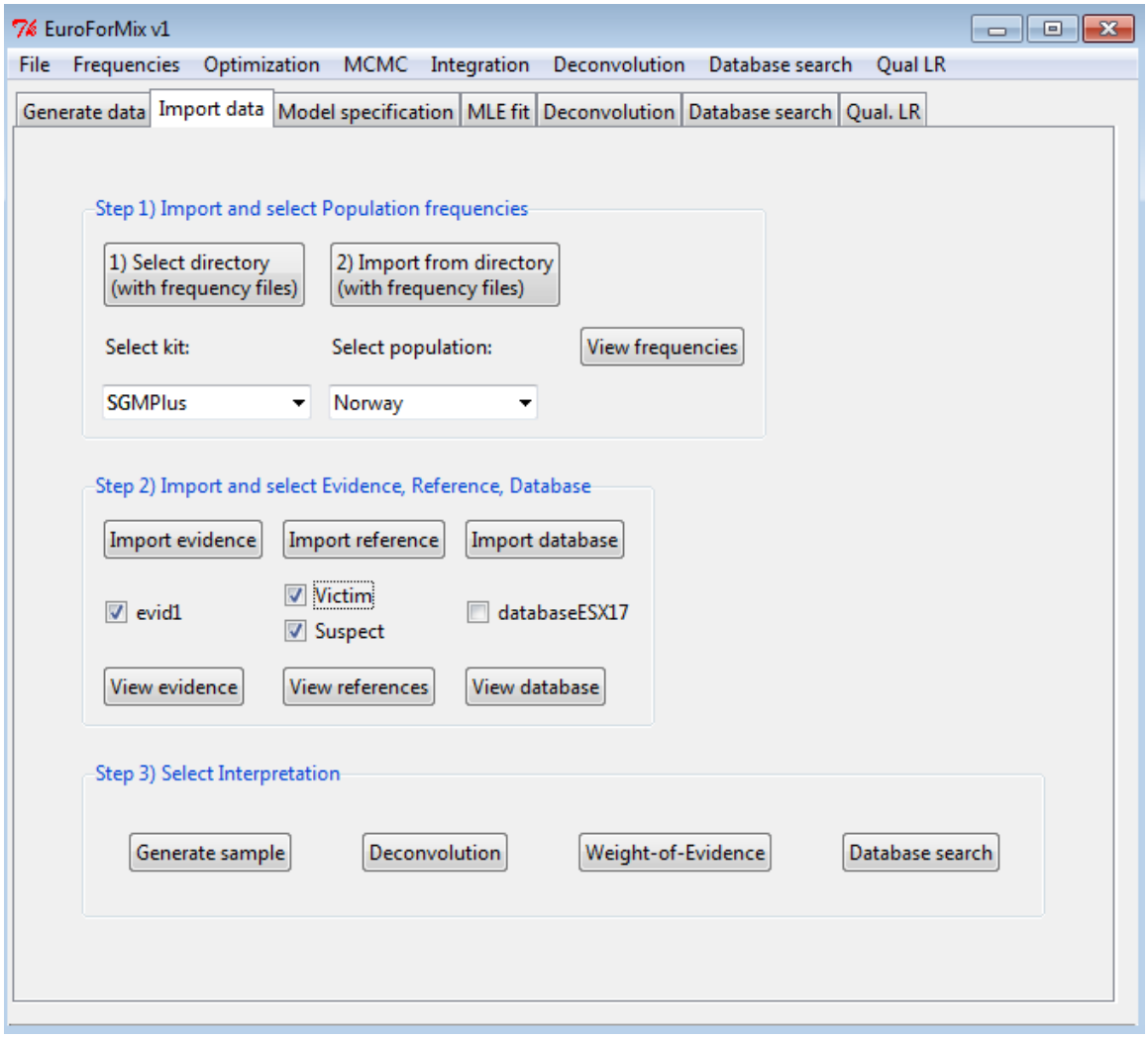


Figure 1: The figure shows the Import data page where the user can import population frequencies, evidence stains, reference profiles and reference databases.

DATA IMPORT:

- **Common** for all files:

  - The extension (denotes file-type) of the file names does not matter. It may also have no extension at all.
  - All imported files must be either comma, semi-colon or tab-separated (‘,’,’;’,’\t’).
  - Required/optional headers (all are capital invariant):
    - “**sample**” is required header for sample(s) name(s).
      - The sample names are NOT capital invariant.
      - If more than one header name contains “**sample**”, it will select the header name which in addition contains “**name**” in the same string.

- "**marker**" is required header for marker name(s).
  - Marker names are capital invariant.
  - If no header is found, the header containing "**loc**" will be used if found.
- "**allele**" is required header(s) for allele-information.
  - This may be a vector ("alleleX1",…,"allelleX10") of any length denoting allele(s) to a given marker for a given sample. Here X1,…,X10 can be anything.
- "**height**" optional header(s) for peak height-information.
  - This may be a vector ("heightX1",…,"heightX10") of any length denoting peak height to the corresponding allele(s) in "allele". Here X1,…,X10 can be anything.

o Note:
- The imported data will use upper-letter of marker-names found in the file.
- All imports are printed out in the terminal (see figure 2). From this, the user may check that the data are imported correctly.

```
[1] "Raw fil import:"
   Sample.Name  Marker Allele.1 Allele.2 Allele.3 Allele.4 Allele.5 Allele.6 Height.1
1        evid1    AMEL        X        Y       NA       NA       NA       NA     2136
2        evid1 D3S1358       14       15     16.0       NA       NA       NA      178
3        evid1    TH01        6        7      9.3       NA       NA       NA      419
4        evid1  D21S11       27       29       NA       NA       NA       NA     1128
5        evid1  D18S51       15       17       NA       NA       NA       NA      467
6        evid1 D2S1338       17       19     20.0       23       NA       NA      290
7        evid1 D16S539        9       10     11.0       12       NA       NA      217
8        evid1     vWA       14       15     17.0       NA       NA       NA     1250
9        evid1 D8S1179       10       13     14.0       15       NA       NA      206
10       evid1     FGA       21       22       NA       NA       NA       NA      664
11       evid1 D19S433       13       14     15.2       NA       NA       NA     1157
   Height.2 Height.3 Height.4 Height.5 Height.6   ADO UD1  X
1      1015       NA       NA       NA       NA false  NA NA
2      2405     1982       NA       NA       NA false  NA NA
3       282     1871       NA       NA       NA false  NA NA
4      1750       NA       NA       NA       NA false  NA NA
5       524       NA       NA       NA       NA false  NA NA
6       619      259      649       NA       NA false  NA NA
7       312      743      619       NA       NA false  NA NA
8       440     1232       NA       NA       NA false  NA NA
9       352      978      827       NA       NA false  NA NA
10      714       NA       NA       NA       NA false  NA NA
11      781      922       NA       NA       NA false  NA NA
```

Figure 2: The figure shows the table format in the importing evidence stain file.

- **Import population frequencies**:

o Requires a separate folder (population-folder) with **only** frequency-files.
o File-format:
- Filename:
  - The name of the filenames **needs** to be in the format: "kit_population.ext", where .ext can be any extension (or it can be missing).
  - kit="kit-name" and population="population name"
  - The kit-name must be consistent with the short-name of the kit instrument. See *?plotEPG* (R-command after loading *gammadnamix* package) for more details.

        - Example of such files can be found in the *FreqDatabases* folder inside the folder *tutorialdata* in the local *gammadnamix* R installation folder.
    - File:
        - First column contains allele-designations (header-name may be anything).
        - Other columns are frequency-information (header-name denotes the locus name and this is converted to capital letters)).
- To import frequencies:
    - Push button "**1) Select directory**" button to select the population-folder with the population frequency files.
    - Push button "**2) Import from directory**" button to import the population frequency files from the selected folder.
    - The drop-down lists are populated
        - It is possible to **add new files** into the selected population-folder **at any time**; push the button once again to include new information to the drop-down list.
- Selection of kit and population:
    - After importing the frequency-files (after pushed button (**2**)), the user may select the wanted kit and population from the two drop down lists at any time* (***but not after a reference-database file has been imported**).
        - This can be useful to see the EPG layout for different selected kits when the 'View evidence' button is pushed.

- **Import Evidence/Reference** sample (see figure 2 and figure 3):

- **Multiple** evidence or reference profiles are **allowed** in each file.
- In evidence files:
    - "height" header is required for analysis: 'Deconvolution', 'Weight-of-Evidence' (continuous model) and 'Database search'. For 'Qualitative LR' this is not required.
- In reference files:
    - "height" header is optional but will not be used further in any analysis.
- Note:
    - The import function will not check whether number of alleles and corresponding peak heights are the same.
    - Loci without any allele-information (i.e. empty or dropped out), are **NOT** imported.

```
[1] "Raw fil import:"
   SampleName  Marker Allele1 Allele2
1      Victim D3S1358    16.0    15.0
2      Victim    TH01     9.3     9.3
3      Victim  D21S11    29.0    27.0
4      Victim  D18S51    17.0    15.0
5      Victim D2S1338    23.0    19.0
6      Victim D16S539    11.0    12.0
7      Victim     VWA    14.0    17.0
8      Victim D8S1179    14.0    15.0
9      Victim     FGA    22.0    21.0
10     Victim D19S433    13.0    15.2
11    Suspect D3S1358    16.0    15.0
12    Suspect    TH01     6.0     7.0
13    Suspect  D21S11    29.0    35.0
14    Suspect  D18S51    11.0    14.0
15    Suspect D2S1338    17.0    20.0
16    Suspect D16S539     9.0    10.0
17    Suspect     VWA    15.0    17.0
18    Suspect D8S1179    10.0    13.0
19    Suspect     FGA    22.0    25.0
20    Suspect D19S433    14.0    14.0
```

Figure 3: The figure shows the table format for the imported reference file.

- **Import Reference Database (**see figure 4):
  - Exactly same format as reference files.
  - Multiple database file may be imported (**must** be done one-at-the-time).
  - **Requires** that population frequencies are imported and selected.
    - **WARNING**: Population frequencies may not be changed again after database importing!
  - Note:
    - The ranking of databases are done over all selected databases.
    - Same samples within a database needs to be in same block but markers within sample can be different orders.
    - Some samples **may** have more/less markers than others (e.g. SGMplus profiles contra ESX18).
      - **Missing markers** for a sample are given with NA.
    - Only markers shared with selected population frequencies are imported.
      - The imported database files may contain different markers.
    - Homozygote genotype may have an empty allele under ‘Allele 2’.
    - The database file may contain **any** number of individuals.
  - Tips:
    - It is more efficient to import several small databases than one big.
      - Time usage to import a database file with 17 markes:
        - 1e6 profiles takes about 131 seconds
          - Requires ~1.3GB memory
        - 5e6 profiles takes about 800 seconds.
          - Requires ~6.1GB memory
    - Save a lot of time and memory by storing a project to file (See File under toolbar). The imported database will be stored very efficiently.

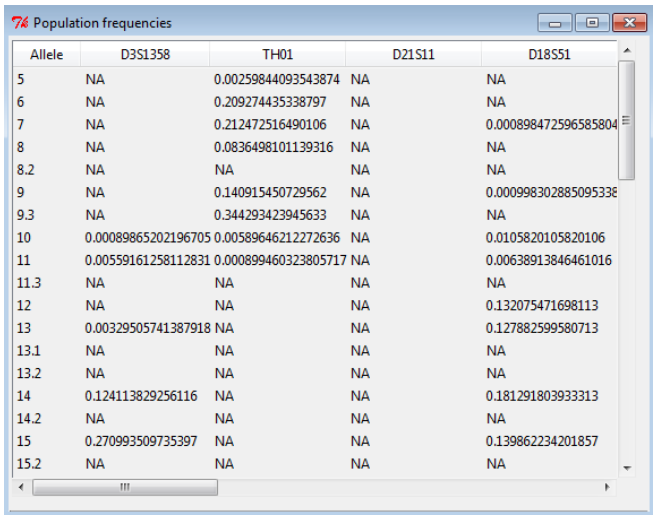
```
[1] "Raw fil import:"
                        Sample.Name   Marker Allele.1 Allele.2
1   00-JP0001-14_20142342311_NO-3241  D3S1358       14       15
2   00-JP0001-14_20142342311_NO-3241     TH01        7      9.3
3   00-JP0001-14_20142342311_NO-3241   D21S11       29       30
4   00-JP0001-14_20142342311_NO-3241   D18S51       13       17
5   00-JP0001-14_20142342311_NO-3241 D10S1248       12       13
6   00-JP0001-14_20142342311_NO-3241  D1S1656       11       14
7   00-JP0001-14_20142342311_NO-3241  D2S1338       17       19
8   00-JP0001-14_20142342311_NO-3241  D16S539       10       11
9   00-JP0001-14_20142342311_NO-3241 D22S1045       15       16
10  00-JP0001-14_20142342311_NO-3241      VWA       17       18
11  00-JP0001-14_20142342311_NO-3241  D8S1179       12       13
12  00-JP0001-14_20142342311_NO-3241      FGA       19       22
13  00-JP0001-14_20142342311_NO-3241   D2S441       11       10
14  00-JP0001-14_20142342311_NO-3241  D12S391       17       18
15  00-JP0001-14_20142342311_NO-3241  D19S433       13       14
16  00-JP0001-14_20142342311_NO-3241     SE33       15       21
17  00-JP0001-14_20142342311_NO-3241     AMEL        X        Y
18  00-JP0002-14_20142342311_NO-3242  D3S1358       15       18
19  00-JP0002-14_20142342311_NO-3242     TH01        6        9
20  00-JP0002-14_20142342311_NO-3242   D21S11       28     31.2
21  00-JP0002-14_20142342311_NO-3242   D18S51       13       18
22  00-JP0002-14_20142342311_NO-3242 D10S1248       13       13
23  00-JP0002-14_20142342311_NO-3242  D1S1656       15     18.3
24  00-JP0002-14_20142342311_NO-3242  D2S1338       25       25
25  00-JP0002-14_20142342311_NO-3242  D16S539       11       13
26  00-JP0002-14_20142342311_NO-3242 D22S1045       15       16
27  00-JP0002-14_20142342311_NO-3242      VWA       14       17
```

Figure 4: The figure shows the table format for the imported reference database file.

VIEW DATA:

- **View frequencies** (see figure 5 for the Norwegian SGMPlus population):
    - Creates a new window which shows the selected population frequencies in a table.
    - If any evidence profiles(s) are selected after evidence-import, the software makes a 'false positive probability' plot for each of the selected profiles.
        - The plot (figure 6) shows the exact probability[1] that a random reference profile (from population) **('false positive probability')** matching at least (2*n-wildcardsize) up to 2*n alleles (MAC) with a **selected evidence** profile. Here **n** is number of considered loci (which are both in evidence and population frequencies) and wildcardsize is the number of allowed mismatches (default is wildcardsize =7).
        - wildcardsize can be changed under "Frequencies" in Toolbar by changing value **Set number of wildcards in false positive match.**
    - Note:
        - Only allele-information in evidence-profiles is used.
        - New alleles which are not found in the selected population are assumed to have allele-frequency 0.

[1] The formula is given in the section 'Exact random allele sharing with evidence stain' under (D) Supplementary.



Allele	D3S1358	TH01	D21S11	D18S51
5	NA	0.00259844093543874	NA	NA
6	NA	0.209274435338797	NA	NA
7	NA	0.212472516490106	NA	0.000898472596585804
8	NA	0.0836498101139316	NA	NA
8.2	NA	NA	NA	NA
9	NA	0.140915450729562	NA	0.000998302885095338
9.3	NA	0.344293423945633	NA	NA
10	0.00089865202196705	0.00589646212272636	NA	0.0105820105820106
11	0.00559161258112831	0.000899460323805717	NA	0.00638913846461016
11.3	NA	NA	NA	NA
12	NA	NA	NA	0.132075471698113
13	0.00329505741387918	NA	NA	0.127882599580713
13.1	NA	NA	NA	NA
13.2	NA	NA	NA	NA
14	0.124113829256116	NA	NA	0.181291803933313
14.2	NA	NA	NA	NA
15	0.270993509735397	NA	NA	0.139862234201857
15.2	NA	NA	NA	NA

Figure 5: The figure shows the viewed frequencies for the Norwegian SGMPlus population.

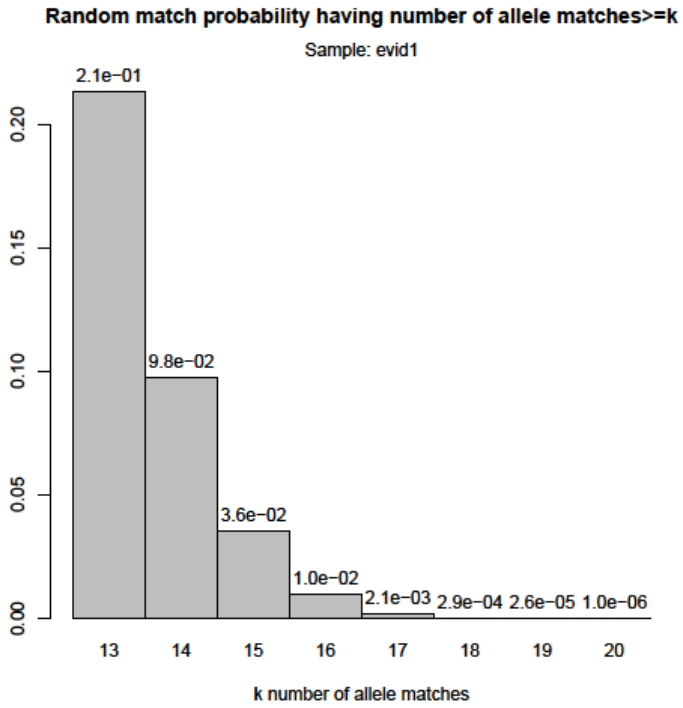


Figure 6: The figure shows the random probability of a match with at least k number of alleles (from a randomly chosen reference profile) compared with the observed alleles in evidence profile (wildcardsize=7).

- **View evidence** (for selected evidence):

    - Prints imported loci, along with allele designations (and peak heights if any) for each selected evidence profile(s) (see figure 7).

```
[1] "Samplename: evid1"
        Allele          Height
AMEL    "X/Y"           "2136/1015"
D3S1358 "14/15/16"      "178/2405/1982"
TH01    "6/7/9.3"       "419/282/1871"
D21S11  "27/29"         "1128/1750"
D18S51  "15/17"         "467/524"
D2S1338 "17/19/20/23"   "290/619/259/649"
D16S539 "9/10/11/12"    "217/312/743/619"
VWA     "14/15/17"      "1250/440/1232"
D8S1179 "10/13/14/15"   "206/352/978/827"
FGA     "21/22"         "664/714"
D19S433 "13/14/15.2"    "1157/781/922"
```

Figure 7: The figure shows the printed alleles and heights in the imported evidence.

- Plot EPG(s) (see figure 8) for each selected evidence profile(s)
  - Requires that the user has imported "Population frequencies".
  - The kit selected under '**Select kit**' denotes the EPG format.
  - Loci in evidence which are **inconsistent** with the ones in selected kit (or missing) are **not shown** in plot.
  - Evidence profiles without peak heights for corresponding alleles are given with peak height equal 1.
  - If reference profiles are imported and selected, they will be labeled together with the peak heights in the EPG plot (as shown in figure 8).
- Note:
  - See *?plotEPG* (R-command after loading *gammadnamix* package) to see which kit-formats that are supported.

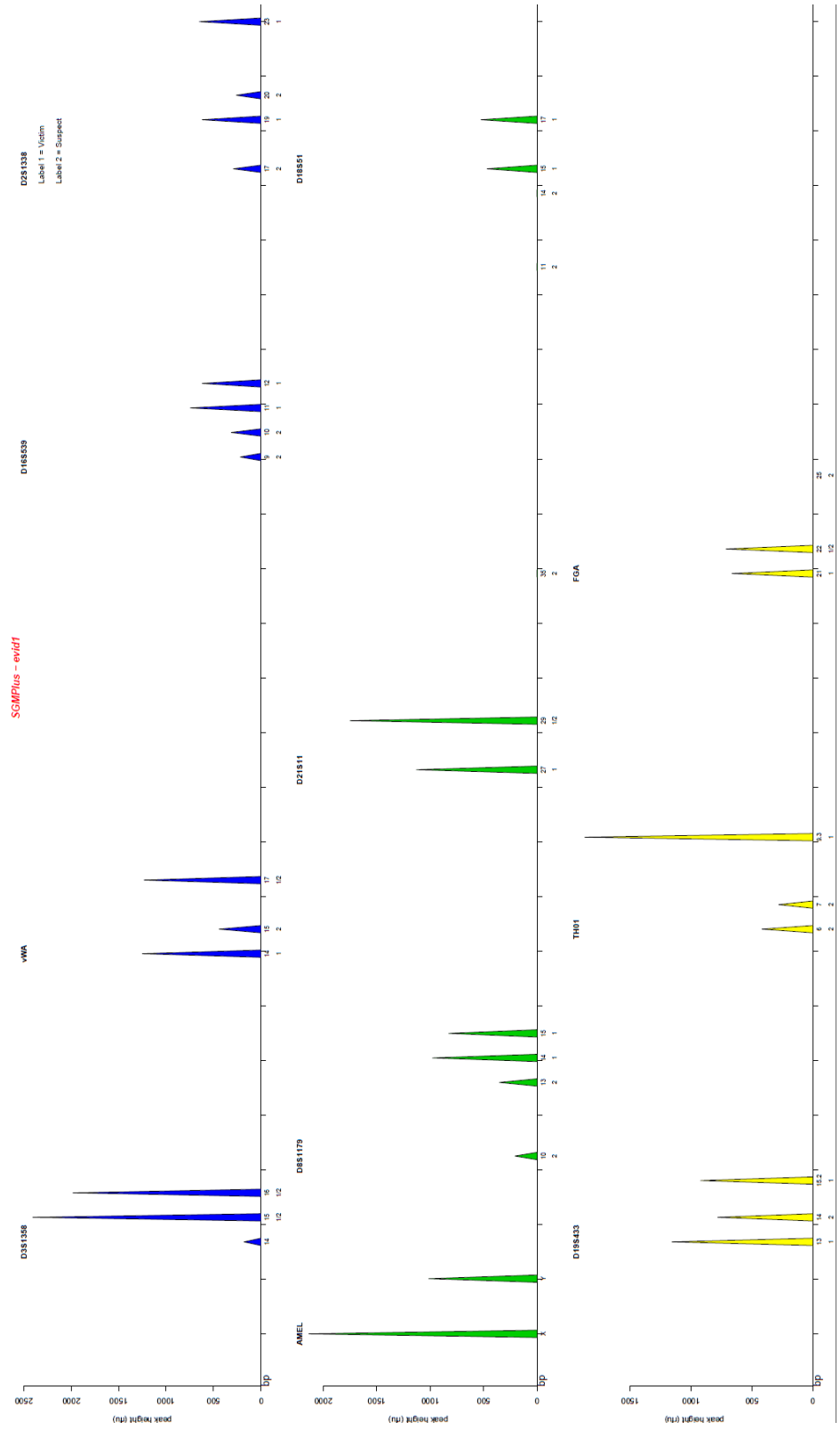


Figure 8: The figure shows the plotted EPG (on the selected SGMPlus kit format) of the imported evidence stain. The labels under the alleles shows the imported and selected reference profiles.

- **View reference** (for selected reference):
  - Prints imported genotypes for each selected reference profile(s) (figure 9).
  - If any evidence profiles(s) are selected after evidence-import, the software counts number of matching alleles (MAC) for each loci of the selected reference profiles, for each selected evidence (figure 10).
    - MAC = number of alleles for the reference which are included in the evidence.
    - nLocs = number of considered loci when counting MAC.

```
         Victim    Suspect
D3S1358 "16/15"   "16/15"
TH01    "9.3/9.3" "6/7"
D21S11  "29/27"   "29/35"
D18S51  "17/15"   "11/14"
D2S1338 "23/19"   "17/20"
D16S539 "11/12"   "9/10"
VWA     "14/17"   "15/17"
D8S1179 "14/15"   "10/13"
FGA     "22/21"   "22/25"
D19S433 "13/15.2" "14/14"
```

-

Figure 9: The figure shows the printed alleles of the imported reference profiles.

```
[1] "Number of matching alleles with samplename evid1:
        Victim Suspect
AMEL        NA      NA
D3S1358      2       2
TH01         2       2
D21S11       2       1
D18S51       2       0
D2S1338      2       2
D16S539      2       2
VWA          2       2
D8S1179      2       2
FGA          2       1
D19S433      2       2
MAC         20      16
nLocs       10      10
```

Figure 10: The figure shows number of matching alleles and total (MAC) between the imported references and selected evidence stain. By combining the observed MAC and figure 6, the random match probability of observing the MAC is useful to provide a 'more meaningful' version of "Random man not excluded"-statistics: The random match probability for Victim (MAC=20) is 1/1000000, while only 1/100 for the Suspect (MAC=16).

- **View database** (see figure 11 for selected database):

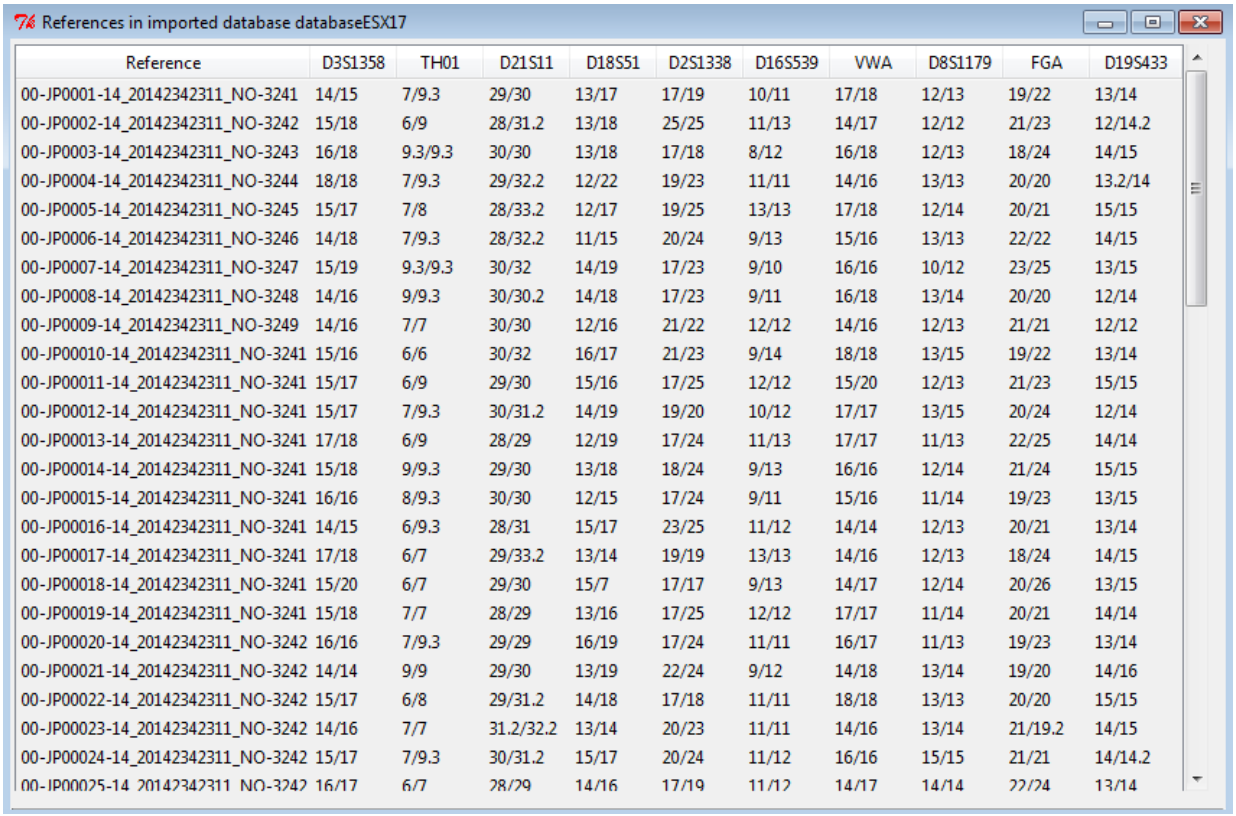
  - Creates a new window (for each selected database) which shows the genotypes for every reference in the database.
    - "NA" means that the genotype of a reference was missing.

  - If any evidence profiles(s) are selected after evidence-import, the software counts the number of matching alleles (MAC) for all references in the database against each of the

selected evidences (see figure 12). The results are shown in a MAC-ranked table in a new window (for each selected database).

- **MAC** = total number of alleles for the reference which are included in the evidence.
- **nLocs** is number of reference-loci which has been used to evaluate the MAC.

- Note:
  - Max number of individuals to view in a database can be changed with selecting **Set maximum view-elements** under "Database search" in toolbar.



Reference	D3S1358	TH01	D21S11	D18S51	D2S1338	D16S539	VWA	D8S1179	FGA	D19S433
00-JP0001-14_20142342311_NO-3241	14/15	7/9.3	29/30	13/17	17/19	10/11	17/18	12/13	19/22	13/14
00-JP0002-14_20142342311_NO-3242	15/18	6/9	28/31.2	13/18	25/25	11/13	14/17	12/12	21/23	12/14.2
00-JP0003-14_20142342311_NO-3243	16/18	9.3/9.3	30/30	13/18	17/18	8/12	16/18	12/13	18/24	14/15
00-JP0004-14_20142342311_NO-3244	18/18	7/9.3	29/32.2	12/22	19/23	11/11	14/16	13/13	20/20	13.2/14
00-JP0005-14_20142342311_NO-3245	15/17	7/8	28/33.2	12/17	19/25	13/13	17/18	12/14	20/21	15/15
00-JP0006-14_20142342311_NO-3246	14/18	7/9.3	28/32.2	11/15	20/24	9/13	15/16	13/13	22/22	14/15
00-JP0007-14_20142342311_NO-3247	15/19	9.3/9.3	30/32	14/19	17/23	9/10	16/16	10/12	23/25	13/15
00-JP0008-14_20142342311_NO-3248	14/16	9/9.3	30/30.2	14/18	17/23	9/11	16/18	13/14	20/20	12/14
00-JP0009-14_20142342311_NO-3249	14/16	7/7	30/30	12/16	21/22	12/12	14/16	12/13	21/21	12/12
00-JP00010-14_20142342311_NO-3241	15/16	6/6	30/32	16/17	21/23	9/14	18/18	13/15	19/22	13/14
00-JP00011-14_20142342311_NO-3241	15/17	6/9	29/30	15/16	17/25	12/12	15/20	12/13	21/23	15/15
00-JP00012-14_20142342311_NO-3241	15/17	7/9.3	30/31.2	14/19	19/20	10/12	17/17	13/15	20/24	12/14
00-JP00013-14_20142342311_NO-3241	17/18	6/9	28/29	12/19	17/24	11/13	17/17	11/13	22/25	14/14
00-JP00014-14_20142342311_NO-3241	15/18	9/9.3	29/30	13/18	18/24	9/13	16/16	12/14	21/24	15/15
00-JP00015-14_20142342311_NO-3241	16/16	8/9.3	30/30	12/15	17/24	9/11	15/16	11/14	19/23	13/15
00-JP00016-14_20142342311_NO-3241	14/15	6/9.3	28/31	15/17	23/25	11/12	14/14	12/13	20/21	13/14
00-JP00017-14_20142342311_NO-3241	17/18	6/7	29/33.2	13/14	19/19	13/13	14/16	12/13	18/24	14/15
00-JP00018-14_20142342311_NO-3241	15/20	6/7	29/30	15/7	17/17	9/13	14/17	12/14	20/26	13/15
00-JP00019-14_20142342311_NO-3241	15/18	7/7	28/29	13/16	17/25	12/12	17/17	11/14	20/21	14/14
00-JP00020-14_20142342311_NO-3242	16/16	7/9.3	29/29	16/19	17/24	11/11	16/17	11/13	19/23	13/14
00-JP00021-14_20142342311_NO-3242	14/14	9/9	29/30	13/19	22/24	9/12	14/18	13/14	19/20	14/16
00-JP00022-14_20142342311_NO-3242	15/17	6/8	29/31.2	14/18	17/18	11/11	18/18	13/13	20/20	15/15
00-JP00023-14_20142342311_NO-3242	14/16	7/7	31.2/32.2	13/14	20/23	11/11	14/16	13/14	21/19.2	14/15
00-JP00024-14_20142342311_NO-3242	15/17	7/9.3	30/31.2	15/17	20/24	11/12	16/16	15/15	21/21	14/14.2
00-JP00025-14_20142342311_NO-3242	16/17	6/7	28/29	14/16	17/19	11/12	14/17	14/14	22/24	13/14

Figure 11: The figure shows the viewed references from the imported ESX17 database which are represented only with SGMPlus loci since the selected kit for the imported frequencies was SGMPlus_Norway.

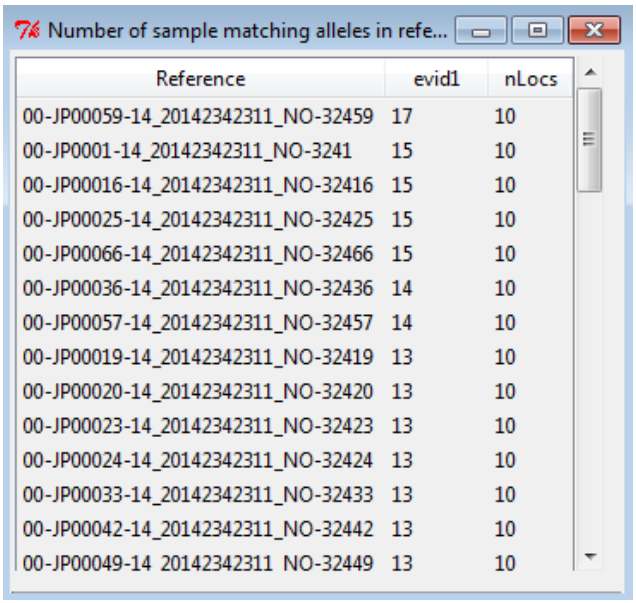


Figure 12: The figure shows the sorted references (in the reference database) with respect to MAC (total number of matching alleles) compared to the selected evidence.

INTERPRETATIONS:

- **Generate sample**:
    - Generates alleles using the population frequencies and draws peak heights for a specified hypothesis using the continuous model as described in the vignette.
    - Requires: Imported population frequencies.
    - Feature: Allele drop-out, Drop-in (with a peak height model) and (n-1)-stutter.

- **Deconvolution**:
    - Deconvolution ranks the most probable combined genotype profiles given **a specified hypothesis** and the Maximum Likelihood Estimates of the parameters in the continuous model (as given in the vignette).
    - Requires: Imported population frequencies and selection of at least one evidence profile with peak height information. References are optional to condition on in the hypothesis.
    - Feature: Model may handle replicates, allele drop-in, drop-out and (n-1)-stutter.

- **Weight-of-Evidence**:
    - Weight-of-Evidence is carried out by comparing the Likelihood Ratio (LR) between the specified hypotheses Hp (prosecution) and Hd (defence) using the continuous model as given in the vignette. There are a number of options as follows:
    - Modules:
        - 1) ‘Continuous LR’ (Maximum Likelihood based)
            - Optimizes (maximum) the model parameters in the continuous model.

        - 2) ‘Continuous LR’ (Integrated Likelihood based)
            - Integrates out the model-parameters in the continuous model.
        - 3) ‘Qualitative LR’ (semi-continuous) – Mirrors the LRmix module.
            - Explores LR as a function of allele dropout probability parameter.

    - Requires:
        - Imported population frequencies, **at least one** evidence profile and **at least one** reference profile (suspect) to weight evidence for. Additional reference profiles are optional to condition on in the hypotheses.
        - ‘Continuous LR’ requires evidence(s) including peak heights, ‘Qualitative LR’ only requires allele data.
    - Feature:
        - The continuous model: Handles replicates, allele drop-in, allele drop-out, (n-1)-stutter and Fst-correction.
        - The semi-continuous model: Handles replicates, allele drop-in, allele drop-out (equal across contributors) and Fst-correction.

- **Database search:**

    - Carries out ‘weight-of-evidence’ tests by comparing the Likelihood Ratio (LR) between the specified hypotheses Hj (reference j in database) and Hd (defence) using the continuous model as given in the vignette.
    - Modules:
        - 1) ‘Continuous LR’ (Maximum Likelihood based)
        - 2) ‘Continuous LR’ (Integrated Likelihood based)
        - 3) ‘Qualitatitve LR’ (Semi-continuous based)
    - Requires: Imported population frequencies, **at least one** evidence profile with **peak height** information and **at least one** reference-database. Reference profiles are optional to condition on in the hypotheses.
    - Feature: Model may handle replicates, allele drop-in, drop-out, (n-1)-stutter and fst-correction.
    - The continuous LR value is shown together with qualitative LR and MAC.

2. Model specification

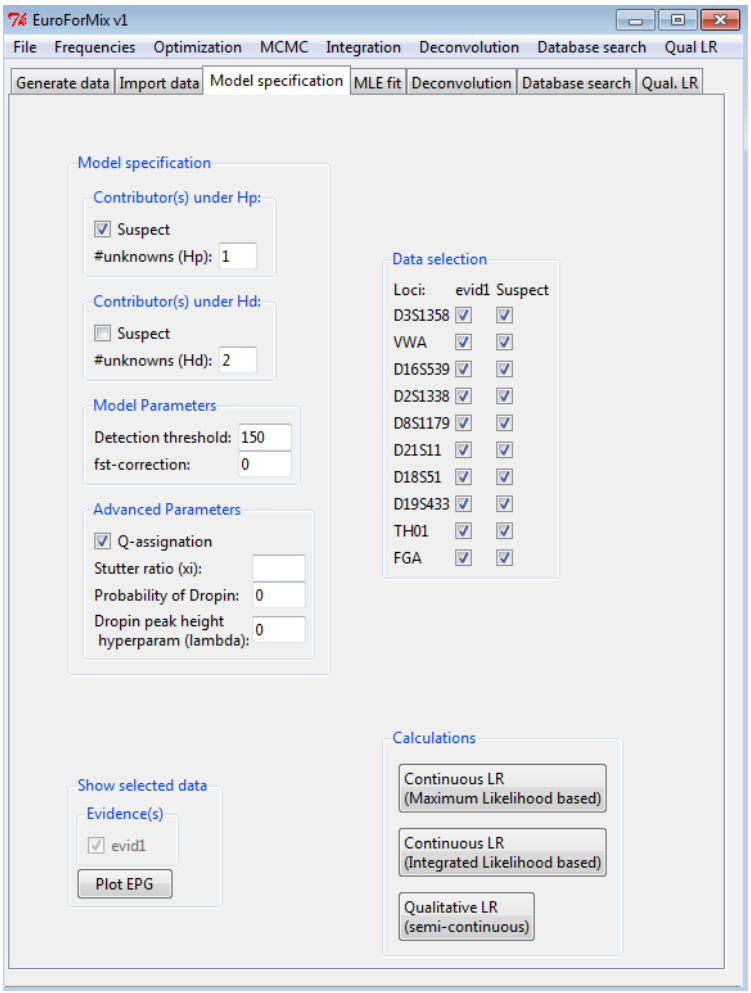


Figure 13: The figure shows the Model Specification page for **Weight-of-Evidence** based on Likelihood Ratio calculation.

MODEL SPECIFICATION

The model specification tab is invoked from several different routes. From the 'Import data' tab the options that can be followed are the buttons: Generate sample, Weight of evidence, Database search and Deconvolution. The effect and properties of each case are as follows:

- **Contributors under Hp**

  - Case: **Weight-of-Evidence** or **'Database search':**
    - User may condition on selected references (from 'Import data') in the hypothesis Hp.
    - #unknowns under Hp: Denotes number of unknown contributors under the prosecution hypothesis Hp.

- Case: **'Database search':**
  - The individual in the reference-database is already included in the hypothesis Hp.
- Case: **Deconvolution** or **'Generate sample'**:
  - This block is not considered, since Deconvolution only considers the model under Hd, and sample generation is carried out only under a specific hypothesis.

- **Contributors under Hd** (same for **all** cases):

  - User may condition on selected references (from 'Import data') in the hypothesis Hd.
  - #unknowns under Hd: Denotes number of unknown contributors under the prosecution hypothesis Hd.
  - Case: **Weight-of-Evidence** or **'Database search'**:
    - References which are conditioned under Hp but not under Hd, will be assumed to be a '**known non-contributor'** under Hd (this is relevant when Fst>0).

- **Model Parameters**:

  - '**Detection threshold'**: [0,->)
    - The limit of detection (LOD) threshold of required allele peak heights. Used to define whether an allele is present in the evidence or not.
    - If peak heights in evidence are lower than the specified threshold, the corresponding alleles (and peak heights) below threshold **are removed** automatically. This may cause some loci to become empty.
    - Not considered if no peak heights are provided in the evidence.

  - **Fst-correction**: [0,1]
    - Assumed co-ancestry parameter assigned in the genotype probability for each contributor in the hypotheses. See vignette for more details.

  - Case **'Database search':**
    - To do a database search with "Continuous LR" Calculations, the allele drop-in probability for the qualitative LR can be changed by **Set drop-in probability for qualitative model** under "Database search" in toolbar (default is 0.05).

  - Case **Generation** and **Deconvolution**:
    - The Qualitative Model Parameters section is removed.

- **Advanced Parameters**

  - **Q-assignation**:
    - If checked, all alleles **not** present in the evidence are designated as a compound allele "99" where its frequency will be given as the sum of the frequencies for all the "non-present" alleles.
    - If unchecked, the original alleles in the population are used as before.

- ‘**Stutter rate’**: [0,1]
  - Only used for ‘Continuous LR’ Calculations.
  - (n-1)-Stutter rate is a constant parameter “**xi**” which denotes the proportion of peak heights from allele ‘a’ which is added to allele ‘a-1’. See vignette for more details.
    - If allele 23 with peak height y_23 is contributed by a contributor and allele 24 did not have any observed peak height, then the stutter contribution to allele 22 from allele 23 will be (**xi *** y_23).
- ‘**Probability of drop-in**’: [0,1]
  - Assumed probability of a random allele drop-in to the evidence at a given locus. See vignette for more details.

- ‘**Dropin peak height hyperparam’**: [0,1]
  - Only used for ‘Continuous LR’.
  - Assumed hyper-parameter to model the peak height of the dropped in allele caused by a ‘random allele drop-in’ if ‘**Probability of drop-in’**>0.
  - See Figure 14 below for more details.

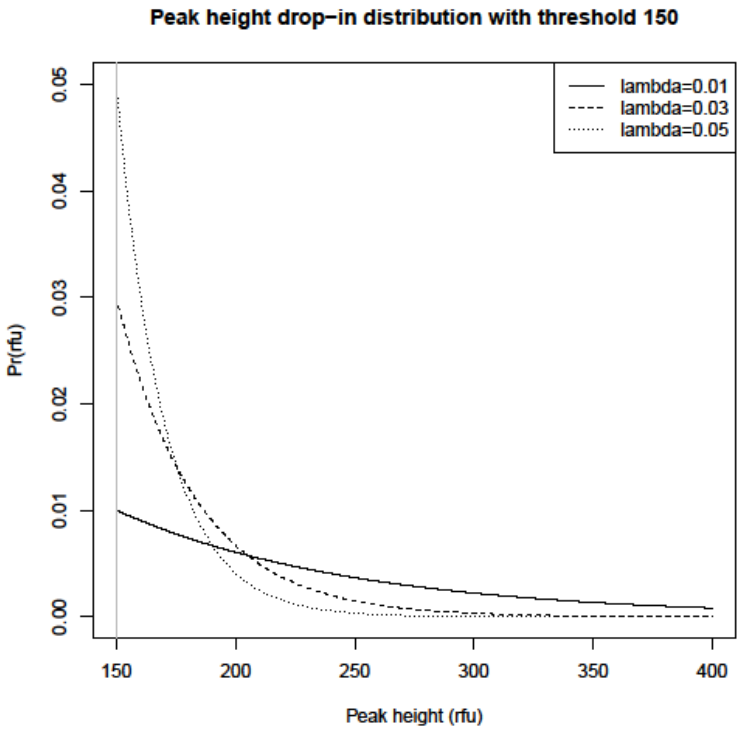


Figure 14: The figure shows the allele peak height drop-in distribution for three values of the lambda hyper-parameter. The distribution is expo(rfu-threshold,lambda) (i.e. shifted exponential).

DATA SELECTION

- **Select/unselect loci**:
    - The user may select or unselect loci for each selected evidence(s) and reference(s) from "Import data"
    - If a locus has been unselected for any of the evidence(s) or reference(s), the unselected locus will not be evaluated at all.
    - Note: There is a limitation of 31 loci that can be selected.

- **Missing data**:
    - Data with missing alleles at any of the loci will automatically be deselected (inactivated) so that the corresponding loci will be unavailable to evaluate.
    - For continuous LR evaluation:
        - If peak heights (in any of the evidence(s)) are missing for any selected locus, the user is given a message to deselect the loci before proceeding further.

- **New alleles**:

If alleles that do not exist in the population allele frequency table occur in the imported evidence or reference profiles, the new alleles are assigned with allele frequency 'freq0'. 'freq0' is equal to the minimum observed allele frequency in the population table if N=0, or 'freq0'=5/(2N) otherwise where N is number of individuals used to create the imported frequency database. This can be changed manually under "Frequencies" in Toolbar.

SHOW SELECTED DATA

- **Evidence(s)**:
    - Shows selected evidence(s) from 'Import data'.
    - All interpretations support **multiple replicates**.
        - Note: All replicates are assumed to have same parameter sets.

- **Plot EPG:**
    - **Prints** the selected evidence sample(s), reference(s) and considered population frequencies which are eventually used for further analysis **out to terminal**.
    - The selected evidence samples are shown in an EPG-plot (go to the RGui Windows, RGraphics device to visualize).
        - Note: Alleles with corresponding peak heights below the specified "Detection Threshold" are removed.

- **'Database(s) to search'** (case**: 'Database search'**)
    - Lists the selected imported reference-database(s) to do the database search for.

CALCULATIONS

- **'Continuous LR (Maximum Likelihood based) '** (case **Weight-of-Evidence** and **'Database search'**):

    - Maximizes the Likelihood of the unknown parameters in the continuous model given the assumed model so they attain maximum values for the specified hypothesis Hd (and Hp in case of Weight-of-Evidence).
        - The optimizer should return a global maximum. However, it may sometimes just return a local maximum. Number of start-points should be increased to ensure that the optimizer finds the global maximum of the Likelihood function. This can be changed under "Optimization" in Toolbar.
    - After calculation, the page 'MLE fit' is visited to present maximized results.

- **'Continuous LR (Integrated Likelihood based)'** (case **Weight-of-Evidence** and **'Database search'**):

    - Instead of optimizing the Likelihood of the unknown parameters, a **multivariate integration** over the unknown parameters are applied both under hypothesis Hp and Hd.
    - The accuracy of the integral depends on the specified '**relative error requirement'** (see vignette for details).
        - Can be changed under "Integration" in Toolbar. Default is 0.005.
    - In the output (see Figure 15), also the relative error of the LR is given in brackets.
    - The integral requires that an **upper boundary** for the parameters mu (mean peak height) and sigma (coefficient of variation of peak heights) are specified. As default these are 21000 and 1, respectively. These values may be changed under "Integration" in Toolbar. See vignette for details.
    - Calculates LR-values directly and avoids visiting the tab 'MLE fit'.
        - Case **Weight-of-Evidence**: A message with the LR pops up after calculation (see Figure 15).
        - Case **'Database search'**: Database search results are shown directly after calculation (goes to tab 'Database search').
    - 'Continuous LR (Integrated Likelihood based)' is not possible for multiple replicates and large number of loci since it doesn't evaluate on log-scale. Use the Maximum Likelihood based method in preference.

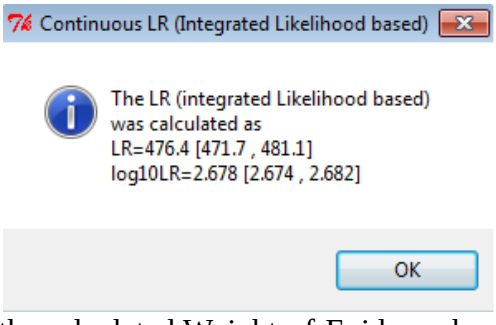


Figure 15: The figure shows the calculated Weight-of-Evidence based the Integrated Likelihood based continuous LR for the specified model in Figure 13.

- **'Qualitative LR (semi-continuous)'** (case **Weight-of-Evidence**)

    - Performs a semi-continuous procedure (mirrors the LRmix module) where the distribution of the 'allele drop-out probability given the number of observed alleles' are utilized to infer a "conservative" LR.
        - The model is purely qualitative which means that it is only based on allele-designation information.
    - Goes directly to page Qual. LR.

- **'Generate sample'** (case **'Generate sample'**):

    - Push **'Generate sample'** button under the 'Import data' tab – this opens the Model specification tab.
    - A dataset (evidence sample and contributing references) will be randomly simulated under the specified model under "Model specification".
    - Reference profiles may be imported and selected as assumed known in the hypothesis.
    - Detection threshold, (n-1)-stutter rate, probability of drop-in and drop-in peak height hyperparam may all be used in the simulation (**Fst** is not used).
    - The unknown contributor profiles under the hypothesis will be randomly generated using the selected population frequencies.
    - The simulated peak heights of the evidence in the dataset are entirely based on the continuous model for assumed values of the model-parameters (**mu,sigma,xi,mx)**. Default these are given as **mu**=1000, **sigma**=0.16, **xi**=0.1, **mx**=(C:1)/sum(C:1), where C is number of contributors.
    - Once the model is completed, push button 'Generate sample' in the 'model specification' tab. The output goes directly to page Generate data. Turn to section 7 for a full description of this page.

3. MLE fit: (‘Continuous LR (Maximum Likelihood based)’)

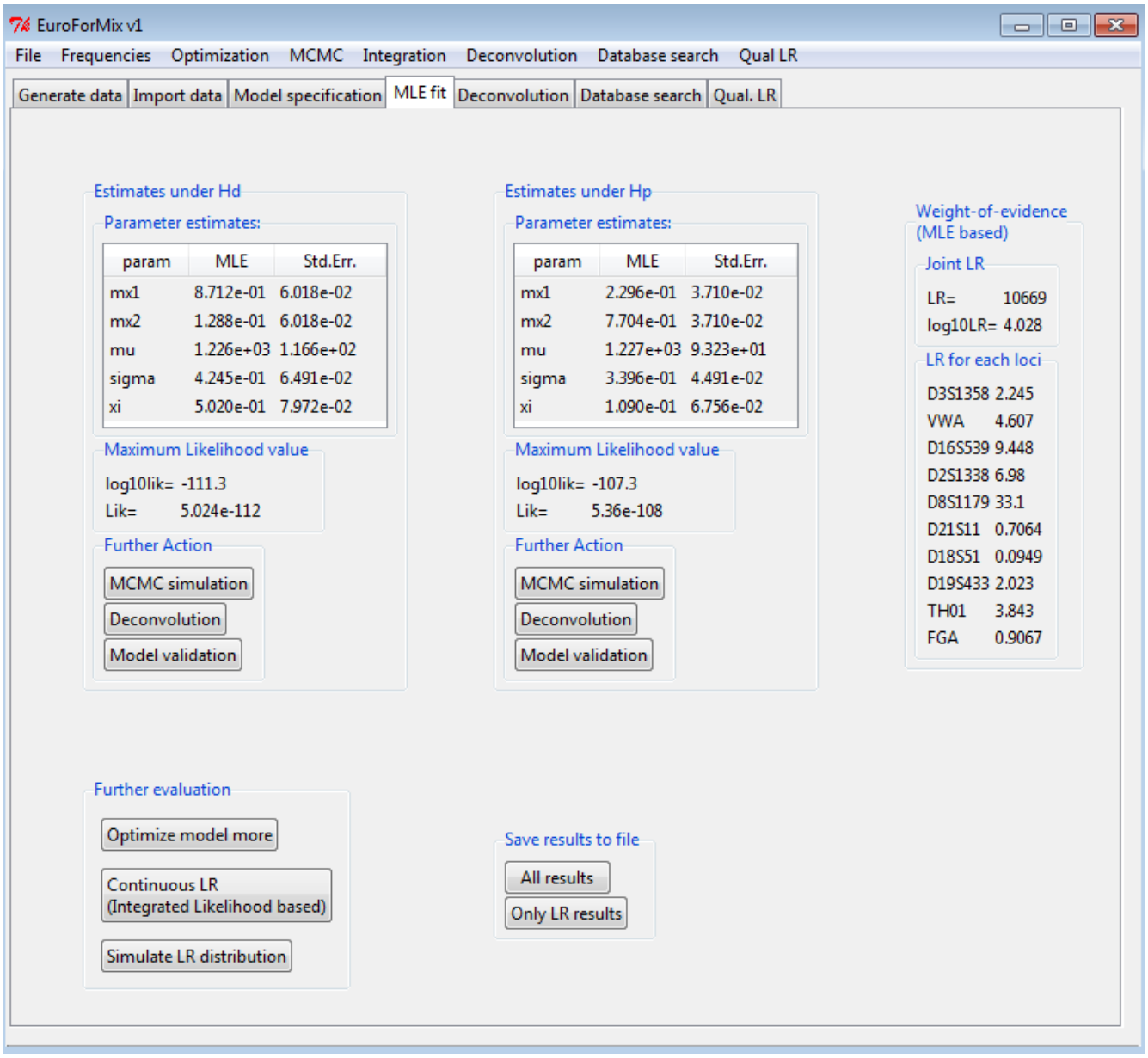


Figure 16: The figure shows the MLE-fit page after running the **continuous LR (Maximum Likelihood based)** calculation (maximizing the continuous model with respect to the unknown parameters for each of the specified hypothesis in figure 13) for **Weight-of-Evidence**.

ESTIMATES UNDER Hd (and Hp for case: **Weight-of-Evidence**)

- **Parameter estimates**:

  - param: The unknown parameters in the model (see vignette for more details).
    - mx_i: Mixture-proportion for contributor 'i'.
    - mu: Mean peak height.
    - sigma: Coefficient of variation for peak heights.
    - xi: (n-1)-Stutter rate (fraction of peak height that are stutter).

  - MLE: The optimized[2] parameters in the model which attains a maximum point of the likelihood function.

  - Std.Err.: The standard error of the parameter estimates in the model (see vignette for details).

- **Maximum Likelihood value**:
  - log10lik and Lik: The ten-logged and the original value of the Likelihood value attained from the optimization[1].

- **Further Action**:

  - **MCMC simulation** (see Figure 17):
    - Performs 'Markov Chain Monte Carlo (MCMC) random walk Metropolis' samples under the desired hypothesis.
      - Uses the mode and the covariance matrix attained from the optimization. See vignette for details.
    - The **first column** in the output shows the estimated posterior distributions for each of the unknown parameters in the model.
    - The **second column** in the output monitors the parameter samples in the simulation.
    - After sampling, the **acceptance rate** of the sampler is printed out to the terminal.
      - Acceptance rate = number of accepted samples divided by number of proposed samples.
      - Tweak '**variance of randomizer**' under MCMC in toolbar to change the acceptance rate[3].
    - User may **change number of required samples** in the simulation under 'MCMC' in toolbar.
    - The **purpose** of the MCMC simulation is to use it as an **exploratory tool** to show:
      - That the optimizer has found the global maximum.
      - The shape of the posterior distribution of the parameters.

[2] This may be only a local maximum point, not the global maximum (i.e. the Maximum Likelihood Estimate). Increase **number of start points** under "Optimization" in Toolbar to ensure a global maximum.

[3] Ideally the acceptance rate should be around 0.2 to ensure that the parameter space has been fully explored.

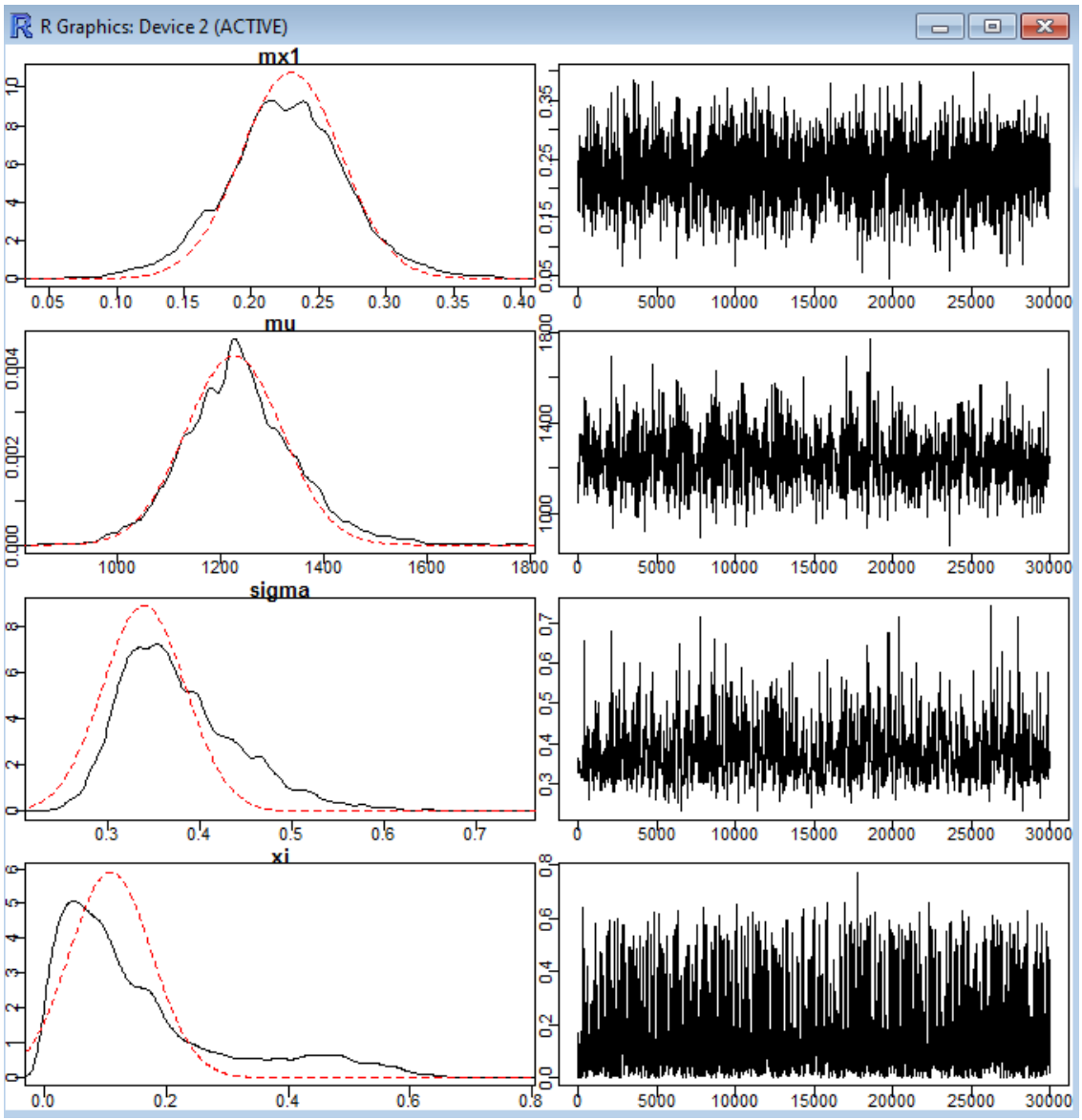


Figure 17: The figure shows the posterior density of the unknown parameters (first column) and corresponding iteration values (second column) from the MCMC method under the hypothesis Hp: "Suspect+1 unknown individual contributes to evidence evid1". The acceptance rate was given as 0.35.

- **Deconvolution**:
  - Performs "Deconvolution" under the desired hypothesis, where the unknown genotypes are ranked with respect to the posterior probability (based on the likelihood function).

- **Model validation** (Figure 18):
  - Uses a statistical hypothesis test to reject if the maximum likelihood fitted model fits the observed peak heights (i.e. whether the gamma model assumption is reasonable).
  - Estimates the cumulative probability of the observed peak heights conditional on the other peak heights (see vignette for more details).

- Uses a one-sample Kolmogorov-Smirnov test to test if the observed cumulative probability deviates significant from the uniform distribution.
  - P-value from the test is printed out to terminal.
  - A textbox is shown when the P-value is lower than the significance level 0.05 (i.e. rejection of assumption).

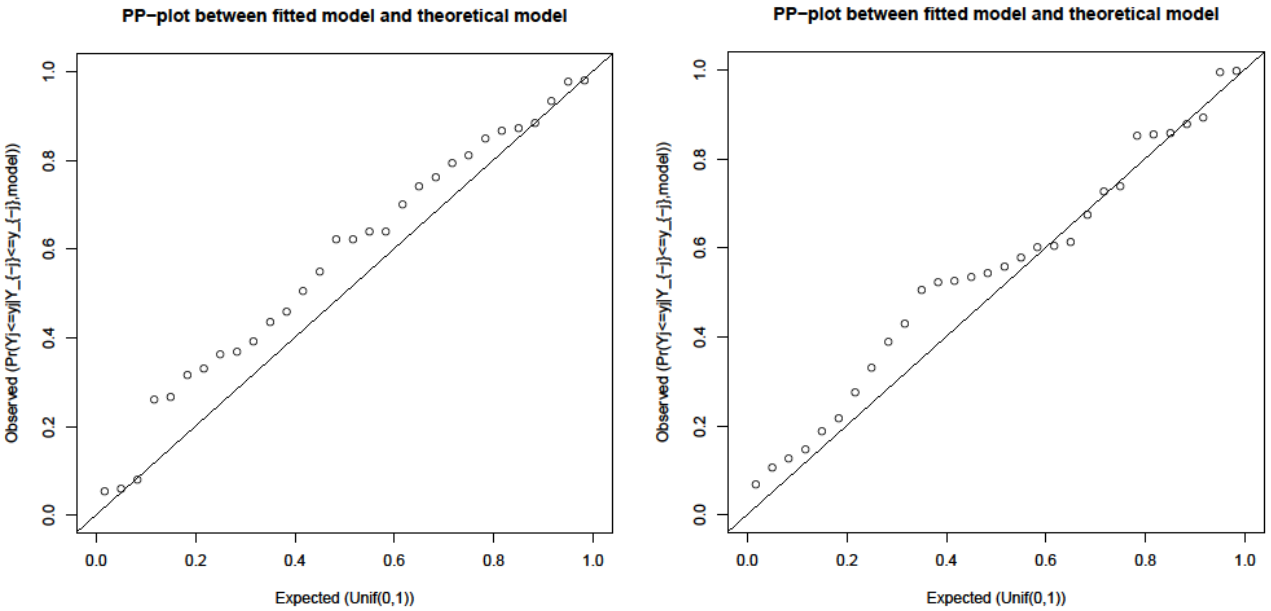


Figure 18: Left subplot shows the "**Model validation**" under Hd with p-value 0.37. Right subplot is "**Model validation**" under Hp with p-value 0.30.

WEIGHT-OF-EVIDENCE (the output of the MLE fit page)

- Description:
  - The Weight-of-Evidence value is the ratio between the likelihoods of the two specified hypotheses Hp and Hd as specified in "Model specification".
  - The Weight-of-Evidence value is based on the continuous model as described in the vignette and handles allele drop-in, drop-out and (n-1)-stutter.
- **Joint LR:**
  - LR: 'Likelihood value under optimization under Hp' divided by 'Likelihood value under optimization under Hd'
  - log10: The ten-logged value of LR.
- **LR for each locus:**
  - The LR for each locus is provided separately (given the parameter-modes under Hp and Hd). See vignette for details.

- Note: At present there is a limitation of 31 loci

FURTHER EVALUATION

- **Optimize model more**:

  - The optimization procedure can be run again with the same specifications as selected in "Model specification" to ensure that a global maximum is attained.
    - It is recommended to do this in order to check that the optimized Likelihood value is not increased further.

- **Database search (case: 'Database search'):**

  - A database search with the specified continuous model will be applied. (See Database search for details.

- **'Continuous LR (Integrated Likelihood based)' (case Weight-of-Evidence)**

  - See CALCULATIONS under section "Model specification".

- **'Simulate LR distribution' (case Weight-of-Evidence)**

  - MCMC simulation will be applied both under Hp and Hd to provide a plot of a "Bayesian" distribution of the LR where the uncertainty of the parameters in the continuous model under both Hp and Hd are taken into account (see Figure 19).
    - Number of samples can be changed with **Set number of samples** under MCMC in Toolbar (default is 10000 samples).

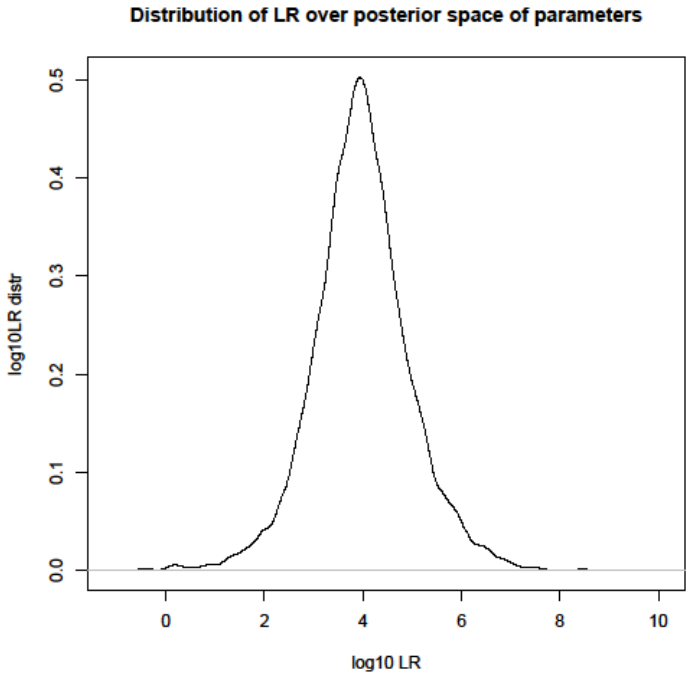


Figure 19: The plot shows the distributed LR where the *a posteriori* density of the parameters in the continuous model under both Hp and Hd are taken into account. *a posteriori* density are simulated using the **MCMC simulation** (Figure 17 shows only Hp)**.**

SAVE RESULTS TO FILE

- **'All results'**:
  - The parameter estimates with corresponding standard deviation errors estimates and the likelihood values will be printed to file for all hypotheses on page (see below).

```
-------Estimates under Hd---------

param-MLE-Std.Err.
mx1-0.87124-0.06018
mx2-0.12876-0.06018
mu-1226.3- 116.6
sigma-0.42447-0.06491
xi-0.50195-0.07972

log10Lik=-111.3
Lik=5.024e-112

-------Estimates under Hp---------

param-MLE-Std.Err.
mx1-0.2296-0.0371
mx2-0.7704-0.0371
mu-1226.65-  93.23
sigma-0.33957-0.04491
xi-0.10902-0.06756

log10Lik=-107.3
Lik=5.36e-108
```

- **'Only LR results'**: **(case Weight-of-Evidence)**
  - The LR calculated values shown in WEIGHT-OF-EVIDENCE will be printed to file (see below).

Marker	LR	log10LR
D3S1358	2.245e+00	0.35113
VWA	4.607e+00	0.66345
D16S539	9.449e+00	0.97536
D2S1338	6.980e+00	0.84384
D8S1179	3.310e+01	1.51979
D21S11	7.064e-01	-0.15094
D18S51	9.490e-02	-1.02273
D19S433	2.023e+00	0.30610
TH01	3.843e+00	0.58467
FGA	9.067e-01	-0.04253
JointMLE	1.067e+04	4.02814

4. Deconvolution:

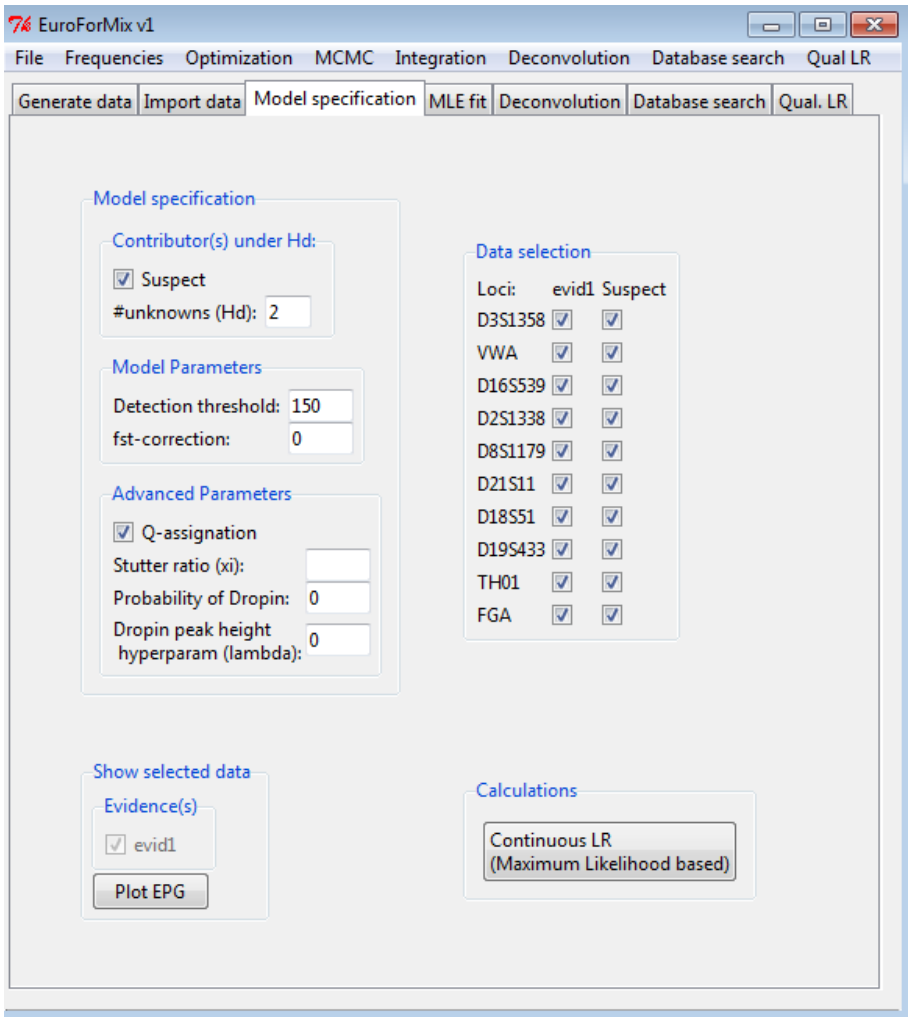


Figure 20: The figure shows the Model Specification page for doing **Deconvolution**. We condition on the suspect, and assume one unknown in the hypothesis. Our model assumes unknown (n-1)-stutter rate, no allele drop-in and no theta-correction.

- Description:

  - Deconvolution is applied for a specific hypothesis Hd as shown in Figure 20.
  - The deconvolution conditions on the optimized parameters (i.e. the MLE fit in Figure 21) for the continuous model.
  - The deconvolution result shows (see Figure 22) a ranked list of the **posterior probabilities** of the combined genotype-profiles (see vignette for details).
  - Since the deconvolution is based on the continuous model it may handle multiple replicates, allele drop-in, drop-out and (n-1)-stutter.

- **Table**:

- The columns in the table (see Figure 22) show the resolved genotype for each contributor in the specified hypothesis (per locus).
- The combined profiles are ranked according to their **posterior probabilities**.
- The ranked elements in the table ensures that the sum of the **posterior probabilities** are at least 0.9999.
  - Can be changed under 'Deconvolution' in toolbar.
- Maximum length of table is default 10000.
  - Can be changed under 'Deconvolution' in toolbar.
- Note:
  - If the parameters in the **MLE fit** are sub-optimized , then the most likely genotypes that result will also be sub-optimal
  - The Q-assignation is recommended since dropped out alleles are treated equally and assigned as "99" in the table.

- **Save table:**

  - The **full** table will be exported to a tabulate-separated text-file.

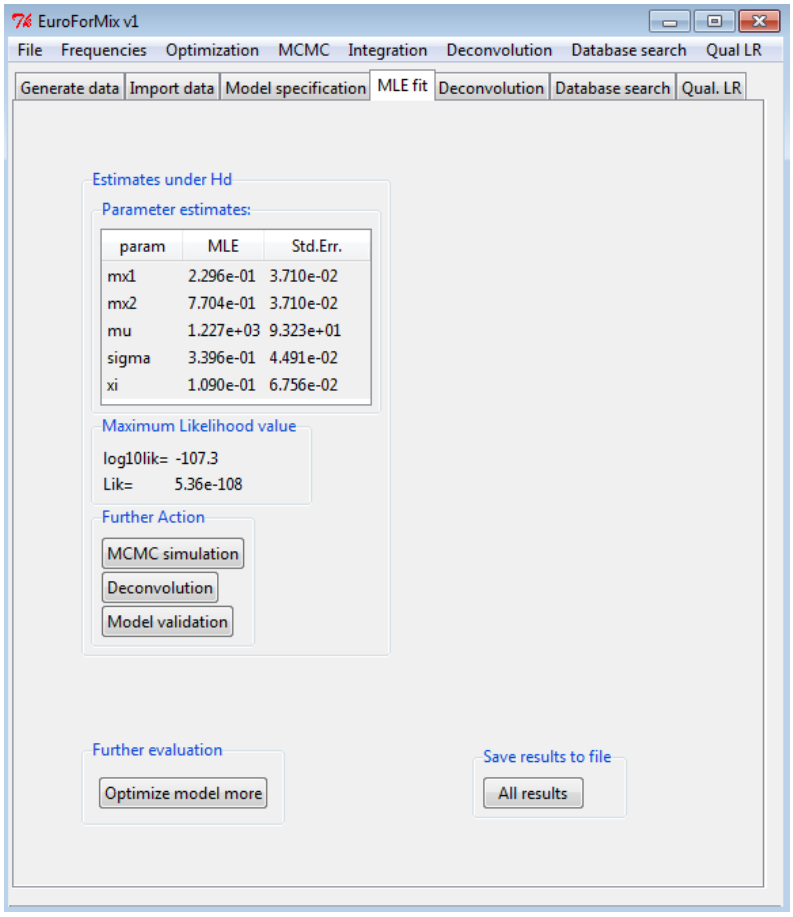
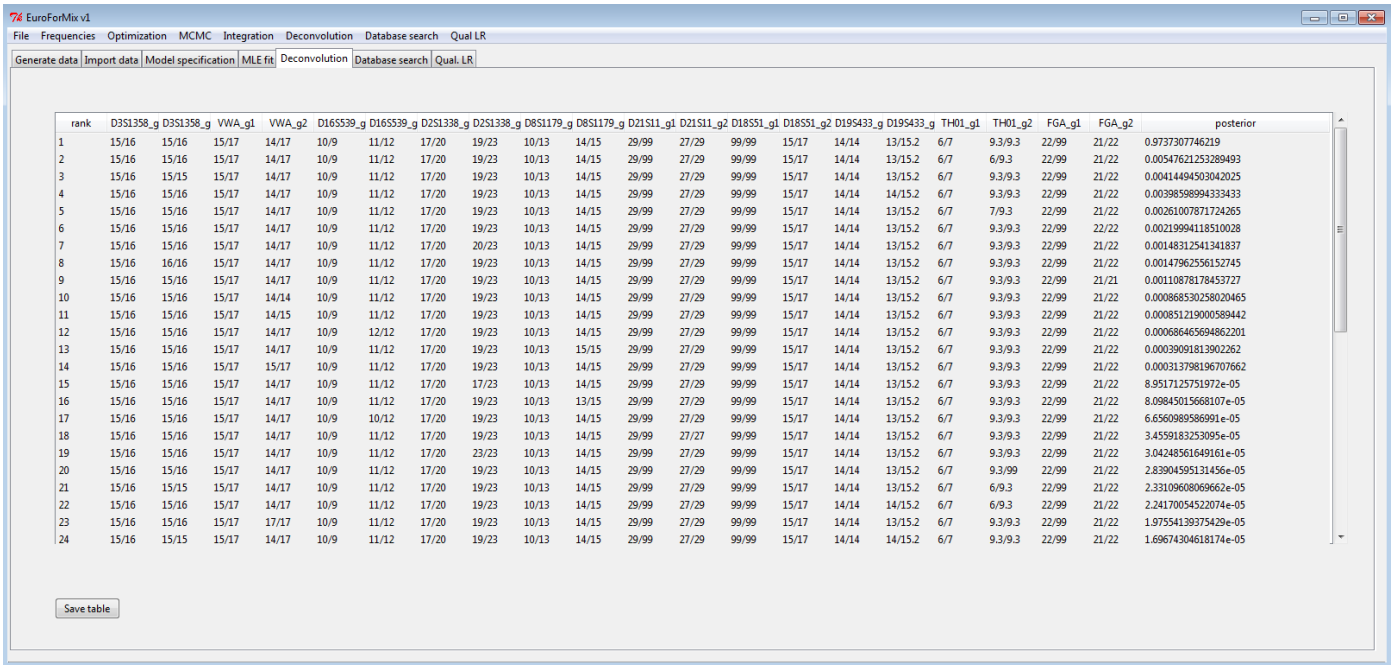


Figure 21: The figure shows the optimized parameters (i.e. the MLE fit) for the continuous model. The fitted model has the same "Further Action" possibilities as for "Weight-of-Evidence" and "Database search" in order to optimize the model.



rank	D3S1358_g	D3S1358_g	VWA_g1	VWA_g2	D16S539_g	D16S539_g	D2S1338_g	D2S1338_g	D8S1179_g	D8S1179_g	D21S11_g1	D21S11_g2	D18S51_g1	D18S51_g2	D19S433_g	D19S433_g	TH01_g1	TH01_g2	FGA_g1	FGA_g2	posterior
1	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.9737307746219
2	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	6/9.3	22/99	21/22	0.00547621253289493
3	15/16	15/15	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.00414494503042025
4	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	14/15.2	6/7	9.3/9.3	22/99	21/22	0.00398598994333433
5	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	7/9.3	22/99	21/22	0.00261007871724265
6	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	22/22	0.00219994118510028
7	15/16	15/16	15/17	14/17	10/9	11/12	17/20	20/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.00148312541341837
8	15/16	16/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.00147962556152745
9	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/21	0.00110878178453727
10	15/16	15/16	15/17	14/14	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.000868530258020465
11	15/16	15/16	15/17	14/15	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.000851219000589442
12	15/16	15/16	15/17	14/17	10/9	12/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.000686465694862201
13	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	15/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.0003909181390226 2
14	15/16	15/16	15/17	15/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	0.000313798196707662
15	15/16	15/16	15/17	14/17	10/9	11/12	17/20	17/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	8.9517125751972e-05
16	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	13/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	8.09845015668107e-05
17	15/16	15/16	15/17	14/17	10/9	10/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	6.6560989586991e-05
18	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/27	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	3.4559183253095e-05
19	15/16	15/16	15/17	14/17	10/9	11/12	17/20	23/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	3.04248561649161e-05
20	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/99	22/99	21/22	2.83904595131456e-05
21	15/16	15/15	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	6/9.3	22/99	21/22	2.33109608069662e-05
22	15/16	15/16	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	14/15.2	6/7	6/9.3	22/99	21/22	2.2417005452207 4e-05
23	15/16	15/16	15/17	17/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	13/15.2	6/7	9.3/9.3	22/99	21/22	1.97554139375429e-05
24	15/16	15/15	15/17	14/17	10/9	11/12	17/20	19/23	10/13	14/15	29/99	27/29	99/99	15/17	14/14	14/15.2	6/7	9.3/9.3	22/99	21/22	1.69674304618174e-05

Figure 22: The figure shows the ranked table of deconvoluted genotype profiles for the unknown major contributor, when conditioning on the suspect profile. The table is ranked with respect to the posterior probability of different combined genotype profiles. Note that the top ranked combined genotype profile is a marked outlier from the other data, which usefully indicates that it is possible to extract the unknown profile (from figure 9 we see that this is a correct extraction).

5. Database search:

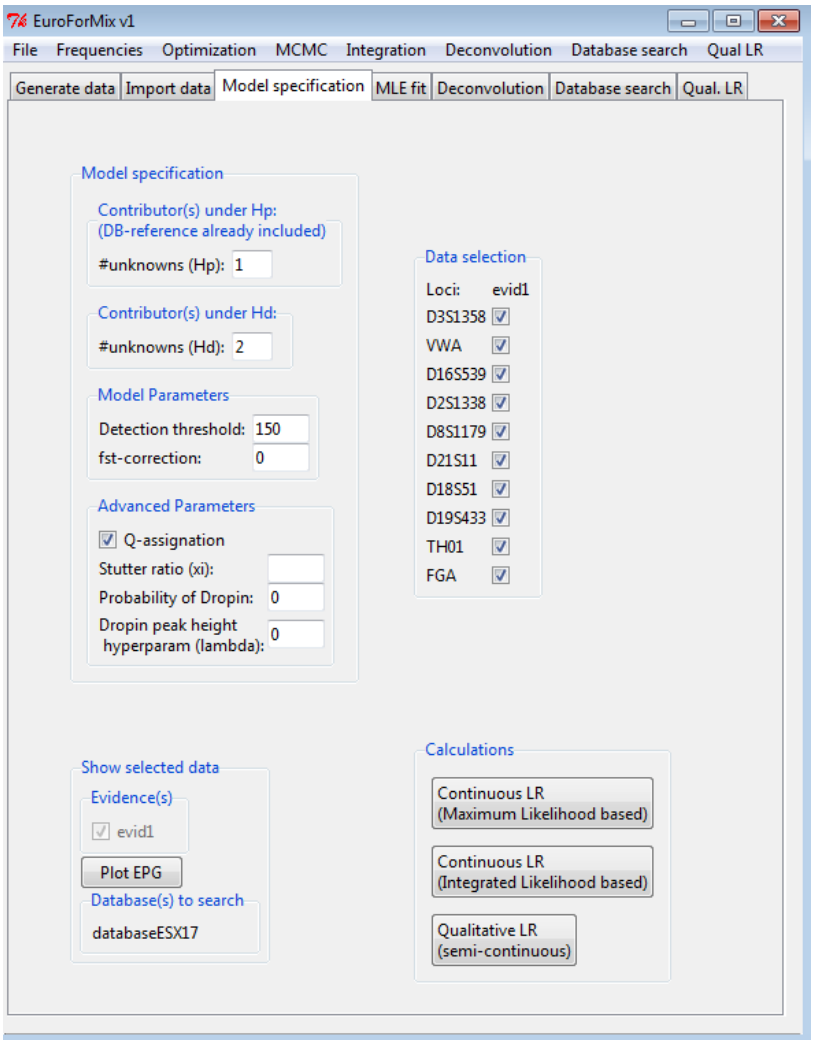


Figure 23: The figure shows the page of the model specification for doing database search on the database file "databaseESX17". Our model assumes no (n-1)-stutter, no allele drop-in and no theta-correction.

- Description:
  - The database to search must be loaded first from the Import data page.
  - Click the database search button from the Import data page which takes you to the Model specification page

  - The 'Database search' is very similar as the Weight-of-Evidence (see Figure 23) with the only difference in that each individual in the reference-database is assumed to be a contributor in the hypothesis Hp. For each individual 'j' in reference-database we calculate a LR-value LRj.

- The user may choose between using peak heights in a 'Continuous LR' (**Maximum Likelihood based** or **Integrated Likelihood based**)' calculation or ignoring the peak heights in a 'Qualitative LR' calculation.

- When selecting 'Continuous LR': (Leads to the MLE fit page)
  - 'Qualitative LR' is always calculated along with the 'Continuous LR' values.
  - The qualitative model assumes an allele drop-out parameter which is estimated.
  - The allele drop-in parameter in the qualitative model is set as default 0.05, but can be changed with "**Set drop-in probability for qualitative model**" under 'Database search' in the Toolbar.
  - No theta-correction is assumed in the qualitative model.
  - If "Continuous LR (Maximum Likelihood based)" calculation is used, the optimized parameters under the Hd -hypothesis are first shown (see Figure 24 where we have assumed no stutter, xi=0 and no allele drop-in).

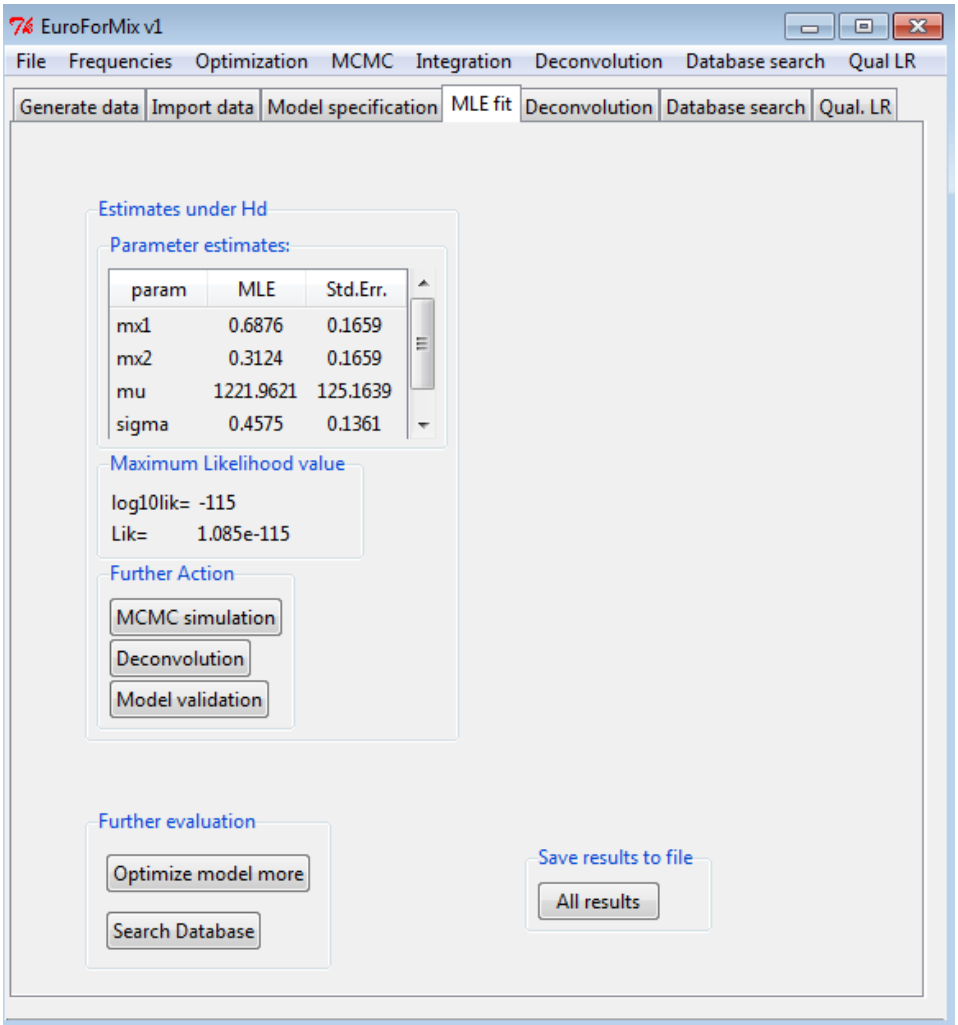


Figure 24: The figure shows the optimized parameters (i.e. the MLE fit) for the continuous model under Hd (with specifications as given in Figure 23). The fitted model has the same "Further Action" possibilities as for "Weight-of-Evidence" and "Deconvolution". The user must push the "**Database search**" button to carry out the actual database searching.

- When selecting ‘Qualitative LR’ from the ‘database search page:

    - The “**Set drop-in probability for qualitative model**” under ‘Database search’ in the Toolbar is ignored.
    - The qualitative model assumes an allele drop-out parameter which is estimated.
    - The ‘Continuous LR’ calculation is ignored.

- Note:

    - The ‘Continuous LR’ calculation is based on the **continuous model** as given in the vignette and can handle allele drop-in, drop-out and (n-1)-stutter.
    - Continuous LR (Integrated Likelihood based) is not possible to use for replicates.
    - The reason for showing the MLE fitted parameters under Hd (see Figure 24) for “Continuous LR (Maximum Likelihood based)” calculation is that the user should have the possibility to check if the parameter estimates under Hd seems reasonable so he can go back and change the model specification.

- **Table** (see Figure 25):

    - ‘**Reference name’** is name of individuals given in the reference-database.

    - The table shows the ranked individuals in the database due to the continuous LR values (**contLR**), qualitative LR values (**qualLR**), number of matching alleles (**MAC**) or number of evaluating loci (**nLocs**).

    - **qual.LR** (Qualitative LR (semi-continuous model))
        - Parameter for dropout probability is based on the median of 2100 samples from the ‘distribution of dropout-probability’.
            - Number of required samples may be changed under ‘Qual LR’ in toolbar.
        - For multiple evidences, the mean of the median is used as the dropout probability parameter.
        - Assumes drop-in probability 0.05 as default. Can be changed under ‘Database search’ in toolbar.
        - Assumes no theta-correction.

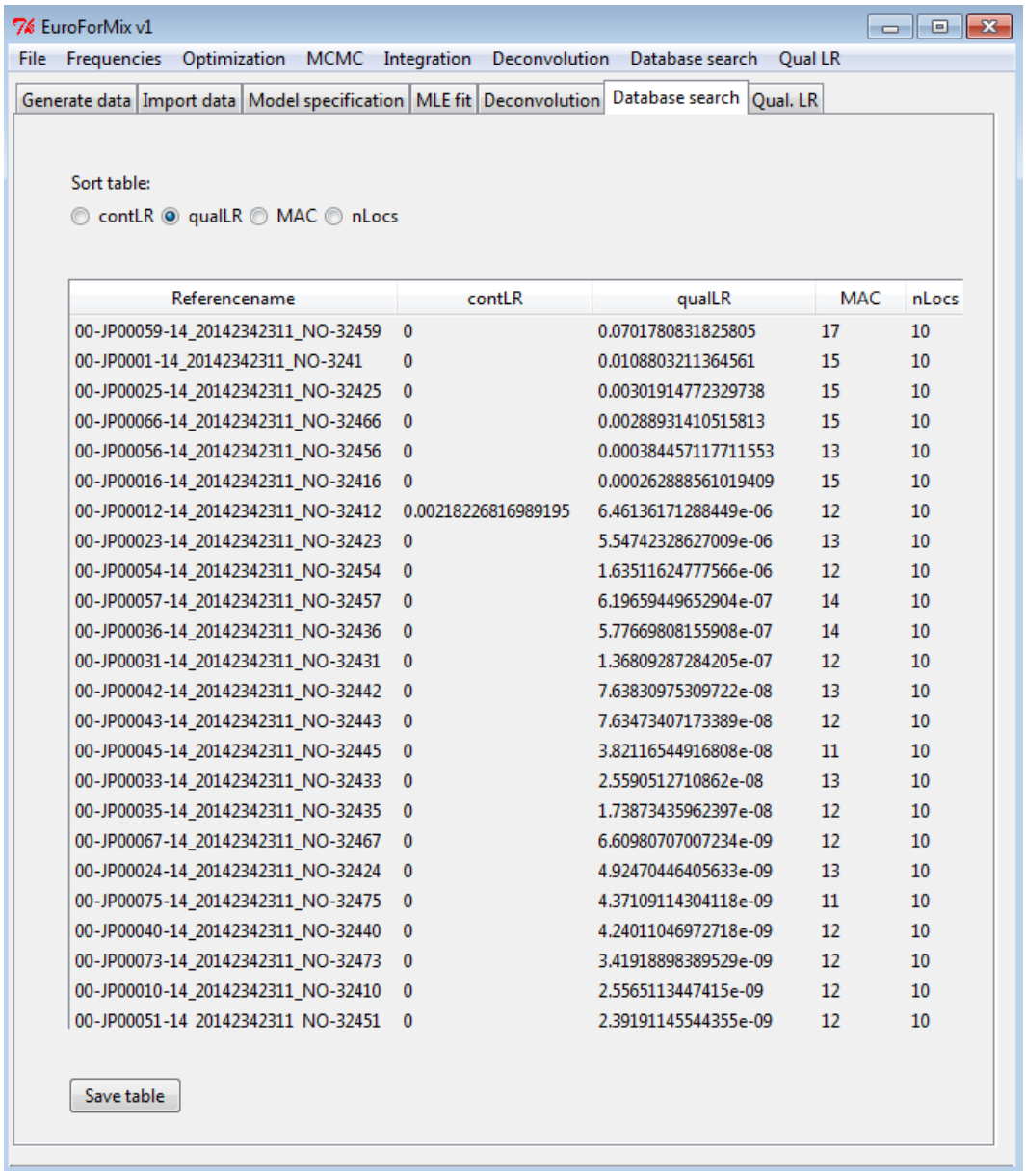
    - **MAC** (Matching allele counter) is number of alleles in the reference-profile which matches the evidence.
        - Note: MAC is summed over the considered evidences.

    - **nLocs** is number of loci in the reference-profile which are used to calculate the contLR,qualLR and MAC.
        - Note: Some references in the database may be missing loci which are presented in the evaluated evidence.
    - Note:
        - Maximum number of elements to view a ‘Database search’ result table is 10000. This can be changed under ‘Database search’ in toolbar.

- Setting Fst>0 may be very time-consuming since we require that individual 'j' is a known non-contributor under Hd, and hence Hd is calculated for each individual in database.
- If no allele drop-in is assumed under the continuous model, **cont.LR** is not calculated for the non-fitting individuals in the database.

- **Save table:**

  - The full table will be exported to a tabulator-separated text-file.



Referencename	contLR	qualLR	MAC	nLocs
00-JP00059-14_20142342311_NO-32459	0	0.0701780831825805	17	10
00-JP0001-14_20142342311_NO-3241	0	0.0108803211364561	15	10
00-JP00025-14_20142342311_NO-32425	0	0.00301914772329738	15	10
00-JP00066-14_20142342311_NO-32466	0	0.00288931410515813	15	10
00-JP00056-14_20142342311_NO-32456	0	0.000384457117711553	13	10
00-JP00016-14_20142342311_NO-32416	0	0.000262888561019409	15	10
00-JP00012-14_20142342311_NO-32412	0.00218226816989195	6.46136171288449e-06	12	10
00-JP00023-14_20142342311_NO-32423	0	5.54742328627009e-06	13	10
00-JP00054-14_20142342311_NO-32454	0	1.63511624777566e-06	12	10
00-JP00057-14_20142342311_NO-32457	0	6.19659449652904e-07	14	10
00-JP00036-14_20142342311_NO-32436	0	5.77669808155908e-07	14	10
00-JP00031-14_20142342311_NO-32431	0	1.36809287284205e-07	12	10
00-JP00042-14_20142342311_NO-32442	0	7.63830975309722e-08	13	10
00-JP00043-14_20142342311_NO-32443	0	7.63473407173389e-08	12	10
00-JP00045-14_20142342311_NO-32445	0	3.82116544916808e-08	11	10
00-JP00033-14_20142342311_NO-32433	0	2.5590512710862e-08	13	10
00-JP00035-14_20142342311_NO-32435	0	1.73873435962397e-08	12	10
00-JP00067-14_20142342311_NO-32467	0	6.60980707007234e-09	12	10
00-JP00024-14_20142342311_NO-32424	0	4.92470446405633e-09	13	10
00-JP00075-14_20142342311_NO-32475	0	4.37109114304118e-09	11	10
00-JP00040-14_20142342311_NO-32440	0	4.24011046972718e-09	12	10
00-JP00073-14_20142342311_NO-32473	0	3.41918898389529e-09	12	10
00-JP00010-14_20142342311_NO-32410	0	2.5565113447415e-09	12	10
00-JP00051-14_20142342311_NO-32451	0	2.39191145544355e-09	12	10

Figure 25: The figure shows the table from the database search with specifications as given in Figure 23 based on '**Continuous LR' (Maximum Likelihood based)"** calculations. The references are sorted due to the qualitative LR's (which assumes allele drop-out probability 0.08 and allele drop-in probability 0.05).

6. Qual. LR:

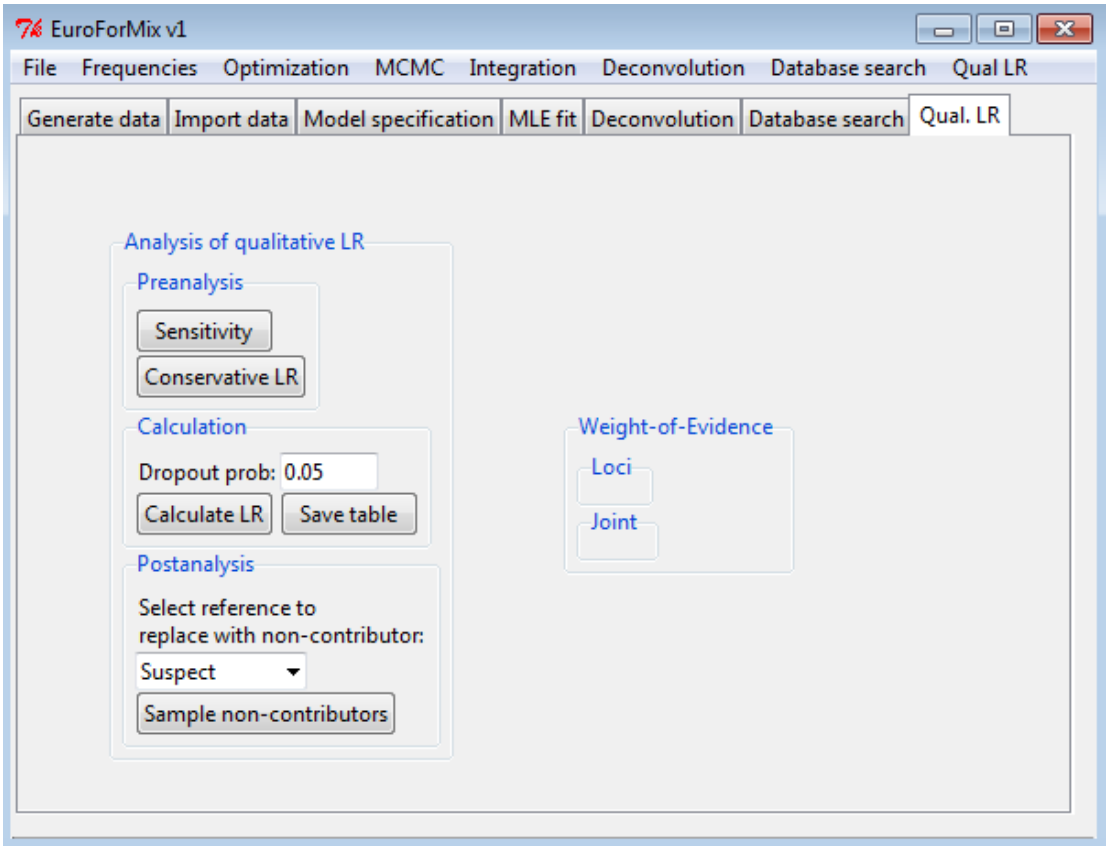


Figure 26: The figure shows the page where the weight-of-evidence evaluation based on the qualitative model is carried out.

- Description:

  - From 'Import data' page, select 'Weight-of-Evidence' button which leads to the 'Model specification' page. Specify the model to test, then select the 'Qualitative LR' button which leads to the 'Qual. LR' page shown in fig. 26.

  - This module samples from the distribution of the '*allele drop-out probability given number of observed alleles*' to evaluate the qualitative LR automatically.
    - Note: the model will crash if there are too many alleles compared to the number of contributors – always check that the model specification is reasonable

  - Also a sensitivity plot as a function of allele-dropout probability and a non-contributor sampling analysis is implemented.

PREANALYSIS

- **Sensitivity:**

- Plots the log10LR as a function of allele-dropout probability (see Figure 27).
  - The upper probability range and number of ticks can be changed under 'Qual LR' in the toolbar.
- Note:
  - Lower range in sensitivity is 1e-6 (something small).

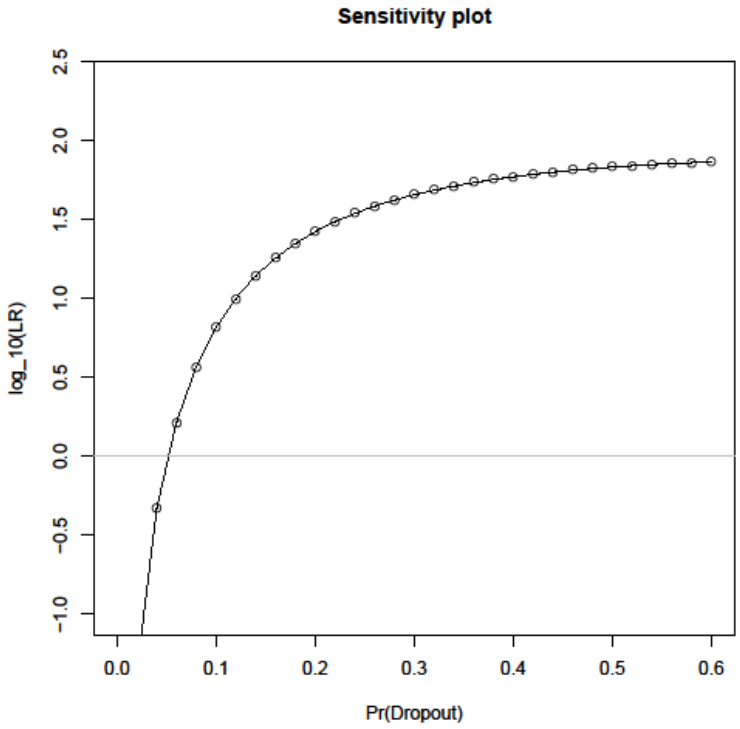


Figure 27: The figure shows the plot of Weight-of-evidence (Likelihood Ratio) as a function of allele drop-out probability.

- **Conservative LR:**

  - By sampling from the "*allele drop-out probability given number of observed alleles in the evidence"*- distribution for the hypothesis Hp and Hd, the most 'conservative' LR (i.e. smallest) is automatically calculated and printed (see Figure 28 and Figure 29).
    - The most "conservative" LR is found by following:
      - Take out the "alpha" and "1-alpha"-quantiles from the simulated 'allele-dropout probability distribution' under both Hp and Hd.
      - The quantile (under both Hp and Hd) which gives the lowest LR is the "conservative LR".
    - The significance level "alpha" is given 0.05 as default.
      - This can be changed under 'Qual LR' in the toolbar.
    - The number of required samples from the 'allele-dropout probability distribution' is given 2000 as default.
      - This can be changed under 'Qual LR' in the toolbar.
    - Note: If no samples are accepted from the allele-dropout probability distribution', an error-message is provided to the user.

- When more evidence samples are imported, the most 'conservative LR' over all samples is considered.
  - The dropout probability quantiles are estimated for each of the evidence samples.

```
[1] "For evidence evid1:"
[1] "Estimating quantiles from allele dropout distribution under Hp..."
        5%        95%
0.01089928 0.23859512
[1] "Estimating quantiles from allele dropout distribution under Hd..."
         5%         95%
0.009575226 0.223586744
         5%  95%
qqhp 0.0110 0.24
qqhd 0.0096 0.22
```

Figure 28: The plot shows the sampled 5% and 95% quantiles of the distribution of the '*allele drop-out probability given number of observed alleles*'.

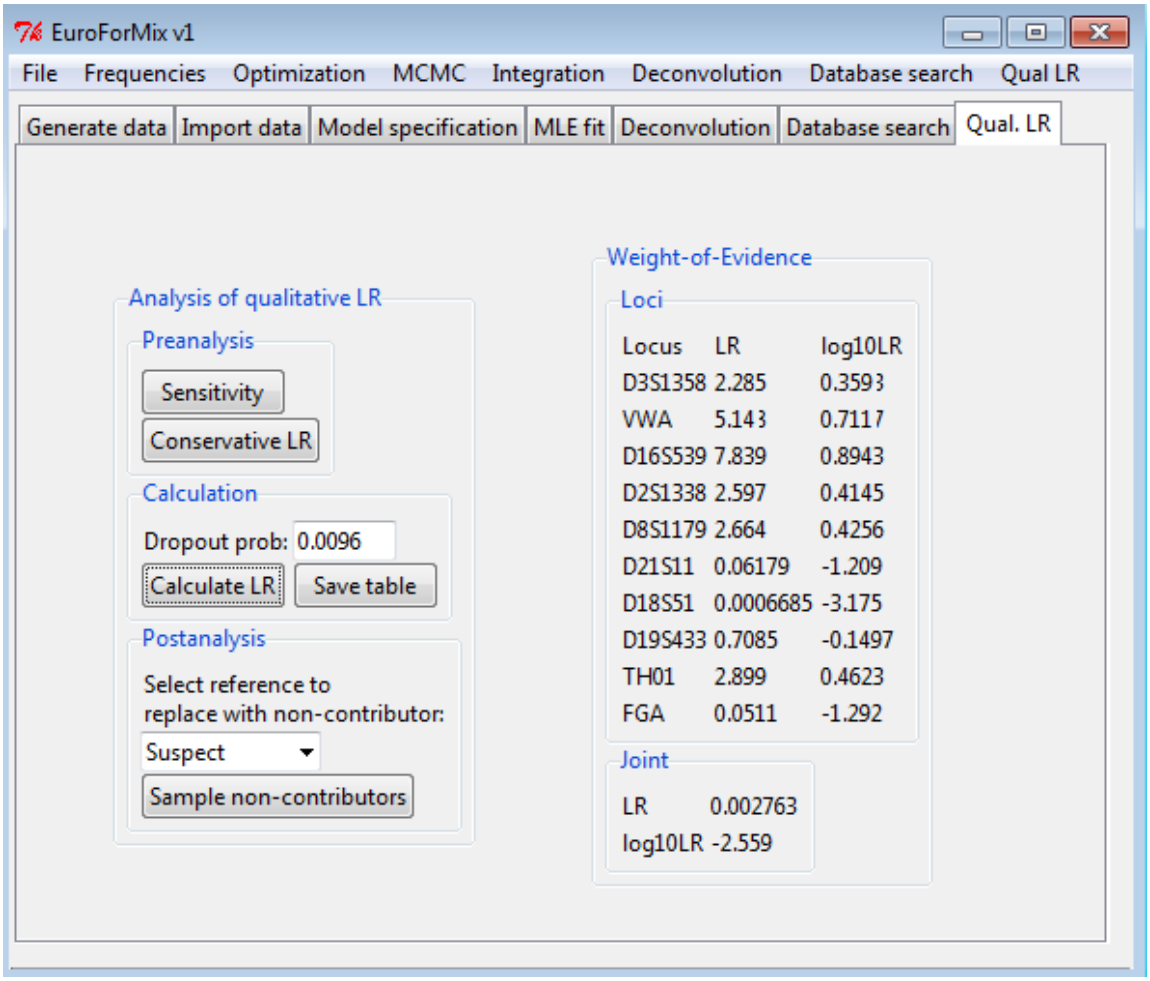


Figure 29: The plot shows the conservative Weight-of-Evidence values (Likelihood Ratios) after pushing the "**Conservative LR**" button. The most conservative estimated allele drop-out probability-quantile from Figure 28 was the 5% quantile under Hd which gave 0.0096. Hence the table in this plot shows the LR inserted for this value.

CALCULATION

- **Dropout prob:**
    - The user may specify the assumed value of the allele dropout-probability.
- **Calculate LR**
    - Instantly calculates the LR for the given user-specified allele dropout probability in "**Dropout prob**".
- **Save table:**
    - Saves the weight-of-evidence calculated LR results to a selected file.

POSTANALYSIS

- **Select reference to replace with non-contributor:**
    - A drop-down list of references which are conditioned under Hp but not under Hd.
- **Sample non-contributors:**
    - Random non-contributor samples are provided by replacing the selected reference (under the drop-down list in the hypothesis Hp) with a random individual from the population and then calculate his LR. A vast amount (default is 1e6) of random non-contributors are simulated to determine the LR distribution of non-contributors.
        - The mean, standard errors of LR and log10LR-quantiles (1%, 5%, 50%, 95%, 99%) are printed out to terminal (see Figure 30).
        - A plot of the cumulative distribution of log10LR will be shown (see Figure 31).
        - Number of non-contributors can be changed under 'Qual LR' in the toolbar.
    - If weight-of-evidence has been calculated:
        - The reporting LR for the "replaced reference" is superimposed as a blue line to the plot (see Figure 31).
        - The discriminatory metric (log10LR-q99%) is printed out to terminal (see Figure 30).
    - Note: Precalculations are always carried out previous to the non-contributor sampling, therefore the number of non-contributors are only limited to make the plot.

```
[1] "Precalculating for non-contributor plot..."
[1] "Simulating 1e+06 non-contributors..."
[1] "Mean of samples = 0.0468785587651348"
[1] "Standard Error of samples = 0.0266497074746912"
        1%         5%        50%        95%        99%
-32.610912 -28.479820 -18.697588  -9.657866  -6.302370
[1] "Discriminatory metric (log10(LR) - q99) = 3.74376679861149"
```

Figure 30: The plot shows the printed non-contributor information to the terminal when replacing the "Suspect" in hypothesis Hp with a non-contributor from the population. Number of simulated non-contributors, mean and standard errors of LR and log10LR-quantiles (1%, 5%, 50%, 95%, 99%) are printed out to terminal (see Figure 30). Also the discriminatory metric, the distance between the observed log10LR for the suspect and log10LR-99%-non-contributors-quantile is given.

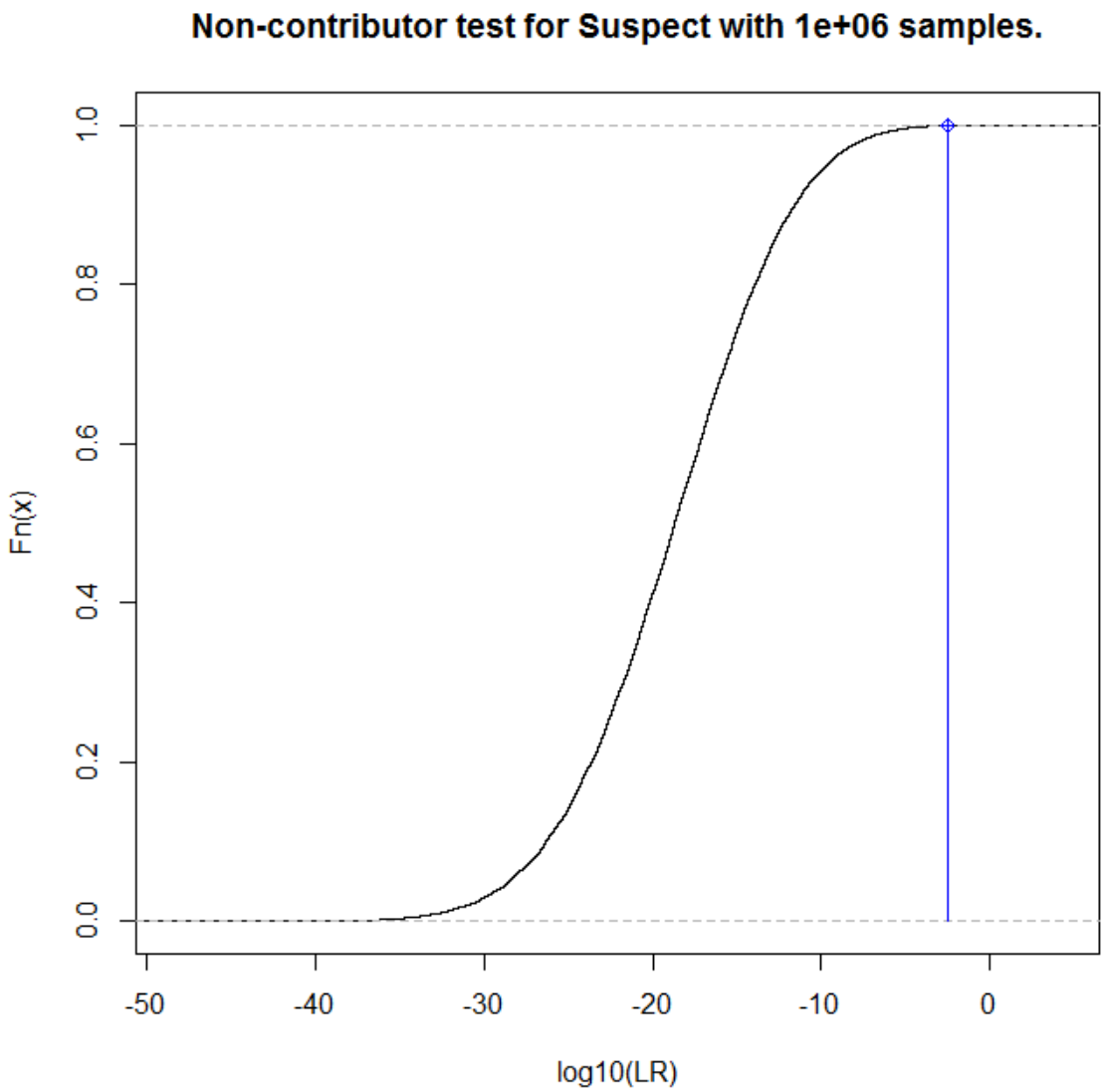


Figure 31: The figure shows a cumulative distribution of 1000000 log10LR of non-contributors, where each sample is based on replacing the "Suspect" in hypothesis Hp with a random man from the population. The reporting LR for the replaced reference (i.e. "Suspect in this case) is superimposed as a blue line to the plot.

7. Generate data:

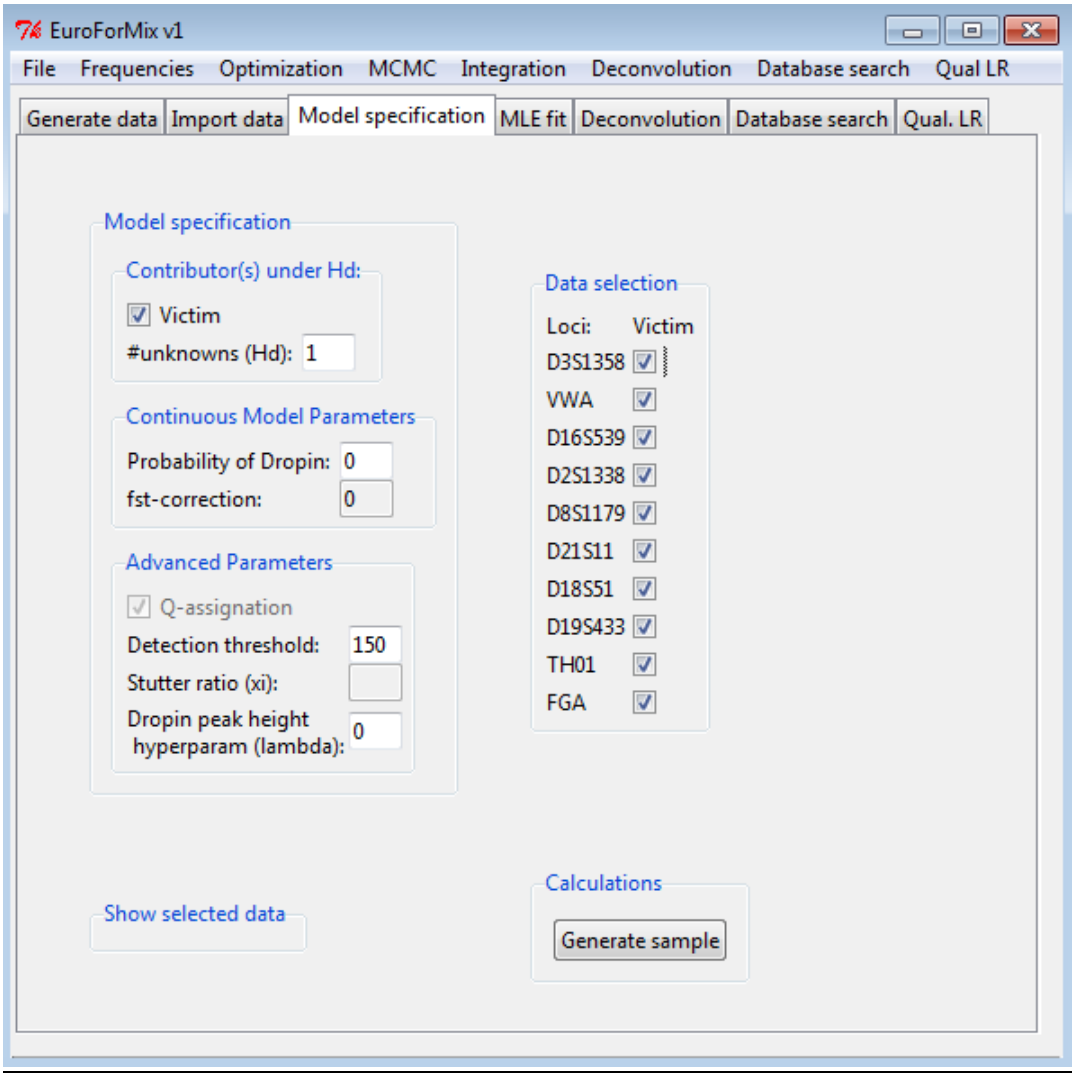


Figure 32: The figure shows the Model specification page for generating allele with corresponding peak heights from the continuous model for a given specified model. From here we will generate data which are contributed from a known Victim profile and an unknown individual. We assume a detection threshold of 150 rfu and no allele drop-in is considered.

- Description:

  - To generate data, the user must first specify the assumptions (hypothesis and known parameters) in the continuous model.
  - The module will generates alleles using the population frequencies and simulates peak heights for a specified hypothesis (see Figure 32) using the continuous model.
  - The generation may simulate allele-dropout, drop-in (with a peak height model) and (n-1)-stutter (see Figure 33).
    - Allele-dropout is indirectly simulated if the peak height is below the defined threshold.

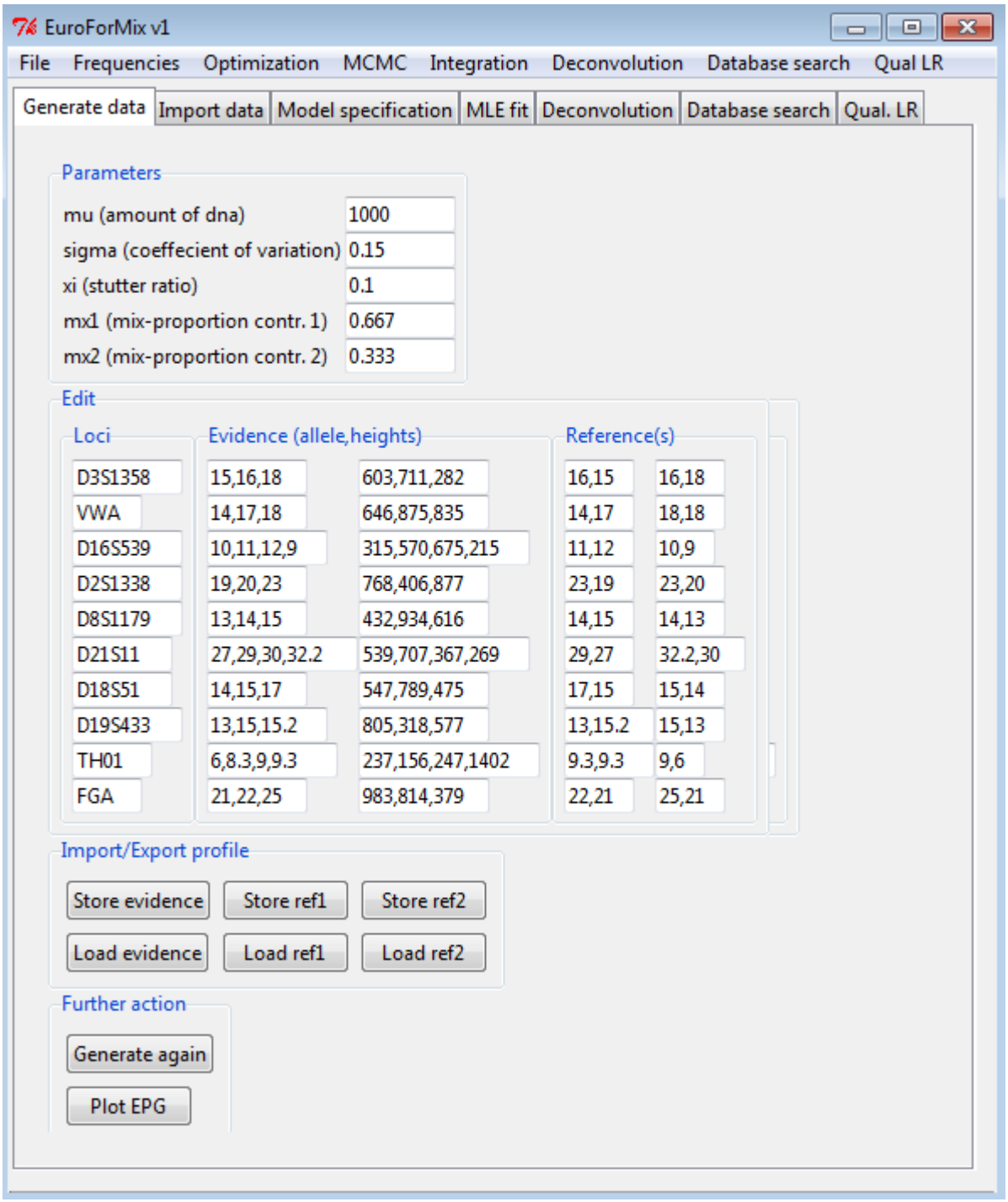


Figure 33: The figure shows the Generate data page which shows the generated alleles and corresponding peak heights (under **Evidence**) for the given selected set of parameters under **Parameters**. The true contributors are given under **Reference(s)**.

- **Parameters**:
    - **mu**: mean peak height
    - **sigma:** coefficient of variance of peak heights
    - **xi:** (n-1)-stutter rate
    - **mx=(mx1,…, mxC):** mixture proportion for contributor 1,..,C.
        - Note: **mx** will be normalized if it's not already.

- **Edit**:
    - **Loci**: Loci name of the population frequency used to generate the dataset.
    - **Evidence**: The allele information is given in the left column while the peak height information is given in the right column. Each element **needs to be** separated with ",".
    - **Reference**: The alleles of the true contributors to the generate evidence is sequentially shown in each column.
    - All the loci names, evidence-allele and heights and reference-alleles may be edited before storing (See Figure 33).

- **Import/Export**:
    - **Save data**:
        - Stores the generated (and possible edited) evidence- or reference-profile to a file.
        - Extension .csv added automatically.
    - **Load data:**
        - Loads profiles from file into the selected entries (evidence or reference).
            - This is useful for generating random evidence samples where loaded references are conditioned on.
        - Note:
            - If any locus is missing from the loaded evidence or reference file, the edit-cell will be empty.
            - The order of the loci in the file does not matter.

- **Further action:**
    - Generate again**:** Make a new simulation of the evidence sample using the selected values of the parameters under **Parameters**.
    - Plot EPG: Plots the generated (and possible edited) evidence in a EPG-plot.
        - It will use the "kit" selected under "Import Data"-page.
        - See ?plotEPG (R-command after loading *gammadnamix* package) to see which kit-formats that are supported in the EPG.

(C) To be implemented in a future version:

- Warning if exp(lik)=0 when lik>-Inf (happens for INT calculations)
- Empty loci will not be removed when imported to the software. They will be considered as a full dropped out loci in the evaluation.
- In deconvolution: Option to only view unknown profiles.
- Non-contributor test for continuous model.
-

(D) Supplementary:

Exact random allele sharing with a evidence profile

Consider marker *i* with mixture $M_i = (A_{i1}, \dots, A_{iI})$ and corresponding allele frequencies $p_{i1}, \dots, p_{iI}$. The number of alleles the defendant shares with the mixture for this marker is denoted Z_i. Let $S_i = p_{i1} + \cdots + p_{iI}$ be the sum of the allele frequencies at marker *i*. Then a direct argument gives (calculations assume HWE and H_d)

$$P(Z_i = 0) = (1 - s_i)^2$$
$$P(Z_i = 1) = 2s_i(1 - s_i)$$
$$P(Z_i = 2) = s_i^2$$

Let $Z = Z_1 + \cdots + Z_I$ be the total number of alleles shared and $\boldsymbol{w} = (w_1, \dots, w_I)$ where $w_i = \{0,1,2\}$ one of these values. Then a for a $k = 0, \dots, I, \dots, 2I$,

$$P(Z = k) = \sum_{\text{all permutations in } \boldsymbol{w}:\sum w_i = k} \prod_{i=1}^{I} P(Z_i = w_i)$$

Here "all permutations" means all possible ordered combinations of the elements in the vector $\boldsymbol{w}$. Note here that RMNE simplifies to $P(Z = 2I) = \prod_{i=1}^{I} P(Z_i = 2)$.