Tutorial for EuroForMix v1

Author: Øyvind Bleka <Oyvind.Bleka.at.fhi.no>

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Part 1: Installation and running program:

1) Install and run R (>=3.0.1) in Windows, Linux or MAC (http://cran.r-project.org/).
    a. Note that this is only tested on a Windows 7 OS (at current moment).
2) Copy and run these commands in the R-software to install the required packages:

```
install.packages("gWidgets");
install.packages("gWidgetstcltk");
install.packages("forensim");
install.packages("cubature");
install.packages("gammadnamix", repos="http://R-Forge.R-project.org");
```

3) Run these commands in the R-software to start the GUI EuroForMix

```
library(gammadnamix);
efm()
```

Part 2: How to use EuroForMix

Get started:

1) Find the R-installation folder:
    a. Find the name of the folder where you installed your R software (I installed v3.1.2).
        i. For instance (in Windows 7), my R version was installed at "C:\Program Files\R\R-3.1.2\" or "C:\Programfiler\R\R-3.1.2\" (for the Norwegian OS version).
2) Find the installation folder of *gammadnamix* and select the folder *tutorialdata*
    a. C:\Program Files\R\R-3.1.2\library\gammadnamix\tutorialdata
    b. Copy this folder to some *easy accessible folder*.

3) Get GUI as top layer (in Windows):
    a. Set the EuroForMix GUI as top layer by using ALT+TAB (at keyboard).

4) Selecting the Working Directory to access the tutorial data:
    a. Click on **File** and then *Set Directory* at the Toolbar.
    b. Find your *easy accessible folder* and select the copied folder *tutorialdata*
    c. Press **OK.**

The basics:

1) Import Population frequencies:
    a. Press **1) Select directory** button.
        i. Find back the folder you selected as Working Directory. Click on the folder and click on the folder *FreqDatabases**.*** Then press **OK** and go back to the GUI.
    b. Press **2) Import from directory** button
        i. Now all population frequency files in the selected folder *FreqDatabases* are loaded into the software.

2) Import Evidences and References:
    a. Press **Import evidence** button.
        i. Click on *stain.txt* and then press **Open**.
            1. The stain evidence[1] is now loaded into the software.
    b. Press **Import reference** button.
        i. Click on *refs.csv* and then press **Open**.
            1. The reference profiles are now loaded into the software.

3) View references and their matching summary against the selected evidence.
    a. Check/select *evid1*.
    b. Check/select *Victim* and *Suspect*.
    c. Press **View references**
        i. A table with the genotypes of the references is printed out to the R-terminal.
        ii. Since the *evid1* stain is selected, a matching summary table between the references and the stain is also printed out to the R-terminal.
            1. MAC is number of matching alleles in to reference profiles to the stain.
            2. nLocs is number of valid markers which are used for the match.

4) View the evidence:
    a. Press **View evidence**
        i. An EPG of the evidence is shown as a plot in the R software.
        ii. The allele-names and corresponding peak heights are printed out to the R-terminal.
        iii. Notice that marker set corresponds with the SGMPlus kit and that the selected references are labeled in the EPG.

5) Selecting a kit and population frequency to use in the evaluations.

[1] The sample were amplified using the PowerPlex® ESX 17 System kit (Promega) with 17.5 µL template and the standard 30 cycle amplification protocol on a GeneAmp® PCR System 9700 (Applied Biosystems). Samples were injected on the Applied Biosystems 3500xl Genetic Analyzer at 1.2 kV for 10 s. The results were analyzed in the GeneMapper® ID-X Software (Applied Biosystems) and the limit of detection (LOD) for alleles was set to 150 RFU.

a. Go to the **Select kit:** - drop-down list and select *SGMPlus*.
b. Go to the **Select population:** - drop-down list and select *UK*.
    i. The frequencies of the SGMPlus typed UK population is now selected.

6) View frequencies and get the false positive match probabilities.
    a. Press **View frequencies**
        i. The imported and selected population frequencies are shown in an own GUI window.
        ii. Go to the R-software frame.
            1. Since *evid1* stain is selected, the software calculates and shows the probability that a random man in the population matches the stain with atleast "k" alleles (i.e. Pr(MAC>=k)). The plot shows this probability as a function of "k" number of matching alleles.
            2. Victim has MAC=20, which means the random match probability of the victim becomes 2.2e-8.
            3. Suspect has MAC=16, which means the random match probability of the suspect becomes 1.3e-3.

7) Saving project for later restoring.
    a. Under **File** in Toolbar, press **Save project**. Name a filename (e.g. "proj"), select a folder where you want to save the project-file and press save.
        i. The project (with all imported data and evaluations) can now be restored by pressing **Open project** under **File** in Toolbar at any time.
        ii. This is useful to quickly restore a project session again after the GUI has been closed.

Weight evidence with a continuous model:

1) Check/select *evid1* and *Suspect* and then press **Weight-of-Evidence**
    a. You then come to the Model specification page.

2) First, specify the contributors under hypotheses Hp and Hd.
    a. Hp :"The suspect profile and 1 unknown individual contributes to *evid1*"
    b. Hd :" 2 unknown individuals contributes to *evid1*"

3) Specify model parameters:
    a. If peak heights are imported in the evaluated evidence, the **Detection threshold** should first be specified same as the peak height threshold when extracting the evidence profile information. Let this be 150 as default.

b. The imported evidence is here not applied with any (n-1) stutter-filter (n is as allele name), and hence we need to assume a (n-1) stutter ratio in the model.
    i. To assume that the (n-1)-stutters in the model has an unknown ratio, the box of **Stutter ratio (xi)** must be empty (as default).
    ii. If the stutter ratio is known, the box of **Stutter ratio (xi)** can contain this known ratio.
    iii. The **Q-assignation** means that non-present alleles in the evidence is assigned as allele Q (i.e. "99") with the allele frequency as sum of all the non-present allele frequencies. This will speed things up if checked, but may lead to some inaccuracy when taking account for (n-1)-stutters.
c. Leave all values as default and press **Plot EPG** to see the evaluating data.

4) Calculate **Continuous LR (Maximum Likelihood based):**
    a. Press **Continuous LR (Maximum Likelihood based)**.
        i. The user is now re-directed to the MLE fit GUI page.
        ii. The software now optimizes the Likelihood (under each hypothesis) as a function of the unknown parameters in the continuous model:
            1. **mx=(mx1,…, mxC):** mixture proportion for contributor 1,..,C.
            2. **mu**: amount of DNA
            3. **sigma:** coefficient of variance
            4. **xi:** (n-1)-stutter ratio
    b. Press the **Optimize model more** button (under *Further evaluation)* to be sure that the Likelihood functions are optimized.
        i. The optimized likelihood values are given under *Maximum Likelihood value*.
    c. Press **Model validation** under *Further action* for each of the hypothesis to test whether the fitted model is not adequate with the observed peak heights.
        i. A Goodness-of-fit test reports a p-value for the test.
            1. This is printed out to terminal.
        ii. A large p-value indicates that the fitted model fits the observed peak heights very well, while small p-values indicates that the fitted model does not fit the observed peak heights very well.
    d. The LR values under *Weight-of-evidence* are based on the optimized likelihood functions. More specific, the **Joint LR** values are the ratio between the optimized likelihood value under Hp and the optimized likelihood value under Hd.
        i. LR for each locus is also conditioned on the optimized parameters.
    e. In order to take the uncertainty of the parameter estimates into account to the LR value, the user may press **Simulate LR distribution** (under *Further evaluation*) in order to simulate 10000 from the LR distribution over the posterior space of the unknown parameters in each of the hypothesis.
        i. This could take a while, depending on number of samples (this can be changed under MCMC in Toolbar).

ii. A density smoothed plot is given the R-software together a range of quantiles printed out to the terminal.
iii. Note:
    1. The user could report the 5% quantile as a conservative LR value (I got log10=2.63).
    2. Note that the **Joint LR** values should lie around the mode of the simulated density. Otherwise, the optimization procedure hasn't reached the maximum likelihood value.

5) Calculate **Continuous LR (Integrated Likelihood based):**
    a. Press **Continuous LR (Integrated Likelihood based)**.
        i. The software now integrates out the unknown parameters in each of the likelihood functions to make a marginalized calculates Likelihood Ratio weight-of-evidence which are independent of the unknown parameters.
            1. A flat prior is considered on all the unknown parameters in the continuous model (see vignette for more details).
        ii. Note:
            1. The integration depends on a relative error parameter which gives to accuracy of the integral. This is default 0.005 but can be changed under **Set relative error requirement** under *Integration* at the Toolbar.
            2. This calculation can be done directly from the Model specification page as well.

Deconvolution:

1) If you followed the steps in Weight evidence with a continuous model:
    a. The user should now be at the MLE fit page.
    b. Remember our hypothesis Hp: "The *suspect* profile and 1 unknown individual contributes to *evid1*", where there is 1 unknown individual in the hypothesis.
    c. Assume that we have gathered extra information to say that we know that the *suspect* reference is a true contributor to *evid1*.
    d. To do deconvolution on the unknown individual based on the fitted model, press **Deconvolution** under the section *Estimates under Hp*.
    e. The user is now re-directed to the Deconvolution GUI page which shows a ranked table of the most probable unknown jointly genotype profiles for the unknown.
        i. Allele "99" means that the allele is not presented in the evidence.
        ii. The **posterior** value is the posterior probability of the jointly combined genotypes presented at each row conditioned on the maximum likelihood estimated parameters (see vignette for more details).

2) If you did not follow the steps in Weight evidence with a continuous model:

a. Check/select *evid1* and *Suspect* and then press **Deconvolution** under the Import data page
    i. You then come to the Model specification page.

b. First, specify the contributors under hypotheses Hd.
    i. Hd :"The suspect profile and 1 unknown individual contributes to *evid1*"

c. Specify model parameters:
    i. If peak heights are imported in the evaluated evidence, the **Detection threshold** should first be specified as the lower peak height limit for the imported evidence. Let this be 150 as default.
    ii. The imported evidence is here not applied with any (n-1) stutter-filter (n is as allele name), and hence we need to assume a (n-1) stutter ratio in the model.
        1. To assume that the (n-1)-stutters in the model has an unknown ratio, the box of **Stutter ratio (xi)** must be empty (as default).
        2. If the stutter ratio is known, the box of **Stutter ratio (xi)** can contain this known ratio.
        3. The **Q-assignation** means that non-present alleles in the evidence is assigned as allele Q (i.e. "99") with the allele frequency as sum of all the non-present allele frequencies.
            C) This should always be used when doing deconvolution.
    iii. Leave all values as default.

d. Press **Continuous LR (Maximum Likelihood based)**.
    i. The user is now re-directed to the MLE fit page.
    ii. The software now optimizes the Likelihood (under Hd) as a function of the unknown parameters in the continuous model:
        1. **mx=(mx1,…, mxC):** mixture proportion for contributor 1,..,C.
        2. **mu**: amount of DNA
        3. **sigma:** coefficient of variance
        4. **xi:** (n-1) stutter ratio

e. Press the **Optimize model more** button (under *Further evaluation)* to be sure that the Likelihood function is optimized.
    i. The optimized likelihood values are given under *Maximum Likelihood value*.

f. Press **Model validation** under *Further action* for each of the hypothesis to test whether the fitted model is not adequate with the observed peak heights.
    i. A Goodness-of-fit test reports a p-value for the test.
        1. This is printed out to terminal.
    ii. A large p-value indicates that the fitted model fits the observed peak heights very well, while small p-values indicates that the fitted model does not fit the observed peak heights very well.

g. To do deconvolution on the unknown individual based on the fitted model, press **Deconvolution** under the section *Estimates under Hd*.
h. The user is now re-directed to the Deconvolution page which shows a ranked table of the most probable unknown jointly genotype profiles for the unknown.
    i. Allele "99" means that the allele is not presented in the evidence.
    ii. The **posterior** value is the posterior probability of the jointly combined genotypes presented at each row conditioned on the maximum likelihood estimated parameters (see vignette for more details).

Weight evidence with a qualitative model:

1) Follow the import steps under The basics.
    a. You should then have the evidence *evid1* and the references *Victim* and *Suspect* imported to the software.
    b. You should have selected the *UK* population frequencies for the *SGMPlus* kit.

2) Also select/check the *victim* in the Import data page. And press **Weight-of-Evidence**.

3) First, specify the contributors under hypotheses Hp and Hd.
    a. Hp :"The *victim* and *suspect* profile contributes to *evid1*"
        i. Change number of unknowns under Hp to *0*.
    b. Hd :" The victim profile contributes and 1 unknown individual contributes to *evid1*"
        i. Under Hd, check/select the *victim* and change number of unknowns under to *1*.

4) Specify model parameters:
    a. We will consider a qualitative model to evaluate the evidence.
    b. If peak heights are imported in the evaluated evidence, the **Detection threshold** should first be specified as the lower peak height limit for the imported evidence. Let this be 150 as default.
    c. Set **Probability of Dropin** to 0.05.

5) Press **Qualitative LR (semi-continuous)** which re-directs the user to the Qual. LR page.

6) Press **Sensitivity**
    a. A plot in the R-software shows the Likelihood Ratio (Weight-of-evidence) as a function of probability of allele dropout (equal for same contributors).
        i. Number of ticks and max probability can be changed under *Qual LR* at the toolbar.

7) Press **Conservative LR**
    a. The 5% and 95% quantiles in the distribution of "allele dropout probability given number of total observed alleles in the evidence" are estimated using 2000 samples.
        i. Number of samples and significance level for quantiles can be changed under *Qual LR* at the toolbar.

b. The estimated quantiles are printed out at the terminal in the R-software.
c. The reporting LR under Weight-of-Evidence uses the allele drop-out probability which gives the smallest LR (to make it conservative in favor of the defendant).

8) Press **Sample non-contributors**
   a. The reference *Suspect* is exchanged with a random non-contributor from the selected population. This is sampled 1e6 times (default) and used to show a cumulative density of theses samples by plotting in the R-software.
      i. The blue line is the Joint log10LR under Weight-of-evidence.

Database search:

1) Follow the import steps under The basics.
   a. You should then have the evidence *evid1* and the references *Victim* and *Suspect* imported to the software.
   b. You should have selected the *UK* population frequencies for the *SGMPlus* kit.

2) Press **Import database,** select the file *databaseESX17.txt* in the *tutorialdata*-folder and press **Open.**
   a. A database with 77 ESX17 typed reference profiles are then imported to the software.
   b. The output on the R-terminal shows that allele 7 in D18S51 and allele 19.2 was in FGA was missing in the population frequencies, but are each assigned as the minimum observed allele frequency.
      i. The allele frequencies are after normalized to have sum equal 1.
   c. Tips:
      i. If a database file contains millions of references, it is very useful to split the file up and import each (split) files separately into the software.
         1. Avoid the limitation of Computer Memory
      ii. If the importing process of a reference database takes long time, the user should save the session as a project (use **Save project** under *File*) to avoid the need of importing the same reference database again.
         1. Stores big databases very efficient.

3) Check/select *evid1*, *Victim* and *databaseESX17* and then press **Database Search**
   a. We will now assume that *Victim* is a true contributor to the evidence.
   b. You then come to the Model specification page.

4) First, specify the contributors under hypotheses Hp and Hd.
   a. Hp :"The Database-reference and the victim profile contributes to *evid1*"
      i. Change number of unknowns under Hp to *0*.
   b. Hd :" The victim profile contributes and 1 unknown individual contributes to *evid1*"
      i. Change number of unknowns under Hd to *1*.
5) Specify model parameters:

a. If peak heights are imported in the evaluated evidence, the **Detection threshold** should first be specified as the lower peak height limit for the imported evidence. Let this be 150 as default.
b. The imported evidence is here not applied with any (n-1) stutter-filter (n is as allele name), and hence we need to assume a (n-1) stutter ratio in the model.
    i. To assume that the (n-1)-stutters in the model has an unknown ratio, the box of **Stutter ratio (xi)** must be empty (as default).
    ii. If the stutter ratio is known, the box of **Stutter ratio (xi)** can contain this known ratio.
    iii. The **Q-assignation** means that non-present alleles in the evidence is assigned as allele Q (i.e. "99") with the allele frequency as sum of all the non-present allele frequencies. This will speed things up if checked, but may lead to some inaccuracy when taking account for (n-1)-stutters.
c. We will now assume that allele drop-in can occur with a given peak height model.
    i. Change **Probability of Dropin** to *0.001* (i.e. the probability of having a allele drop-in event to a particular marker).
    ii. Change **Dropin peak height hyperparam (lambda)** to *0.014 (*i.e. the parameter to a shifted exponential density starting from the detection threshold 150 rfu).
        1. Only small peaks up to around 400 rfu are probable.

6) Press **Continuous LR (Integrated Likelihood based).**
    a. The software now integrates out the unknown parameters in each of the likelihood functions to make a marginalized calculates Likelihood Ratio weight-of-evidence which are independent of the unknown parameters.
        i. Note:
            1. A flat prior is considered on all the unknown parameters in the continuous model (see vignette for more details).
            2. The integration depends on a relative error parameter which gives to accuracy of the integral. This is default 0.005 but can be changed under **Set relative error requirement** under *Integration* at the Toolbar.
    b. The user is now re-directed to the Database search page which shows a sorted table of the references in the imported database.
        i. The sorted table can be based on the continuous LR, qualitative LR, Number of Matching Alleles (MAC) or number of evaluated loci (nLocs).
    c. Go back to the Model specification page (by clicking).

7) Press **Continuous LR (Maximum Likelihood based)**.
    a. Press the **Optimize model more** button (under *Further evaluation)* to be sure that the Likelihood function is optimized.
        i. The optimized likelihood values are given under *Maximum Likelihood value*.
    b. Press **Model validation** under *Further action* for each of the hypothesis to test whether the fitted model is not adequate with the observed peak heights.
        i. A Goodness-of-fit test reports a p-value for the test.

1. This is printed out to terminal.

ii. A large p-value indicates that the fitted model fits the observed peak heights very well, while small p-values indicates that the fitted model does not fit the observed peak heights very well.

c. Press **Search Database** under *Further evaluation* to do the database search.

d. The user is now re-directed to the Database search page which shows a sorted table of the references in the imported database.

i. The sorted table can be based on the continuous LR, qualitative LR, Number of Matching Alleles (MAC) or number of evaluated.