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# Interpretation of Complex STR Results Using the Forensim Package

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# 1 The forensim package

## 1.1 Overview. Documentation


forensim is an -package dedicated to facilitate the statistical interpretation of forensic DNA evidence. It also provides simulation tools made to mimic data from casework. A detailed description of forensim is given in the package tutorial, available from: <http://forensim.r-forge.r-project.org/>. The present tutorial aims at describing one particular module of Forenim, LRmix, which allows to calculate the likelihood ratio for complex STR profiles.


**A note on notation** A few typographical conventions are used in this tutorial: different colours are used for the **R commands** and for **the R results**. A verbatim font is used for **R commands**.

## 1.2 Software installation

Before we start, make sure you have installed R properly.

### 1.2.1 Install the R software

The  software is available from the Comprehensive R Archive Network (CRAN). Hereafter we explain how the software can be installed:

- Go to <http://www.r-project.org/>
- In the Getting Started tab, go to : DownloadR
- Choose a CRAN mirror (preferably one close to where you live)
  - Argentina: <http://mirror.fcaglp.unlp.edu.ar/CRAN/>
  - Netherlands: <http://cran.xl-mirror.nl/>
- Dependent on which operating system (computer) you use, click on one of the links: Linux, MacOS X or Windows. For Windows:
  - Click the link base
  - Click the link Download R 2.13.1 for Windows, run the file and the installation program will start.
- Click on R-2.13.1.exe to install the set-up file
- After installation, a blue coloured icon on your desktop, click on the icon to launch an  session (Figure 1).

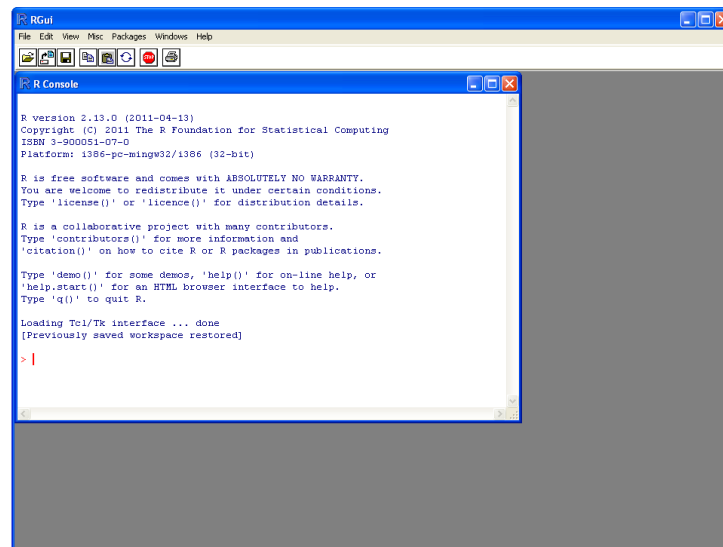



Figure 1: R session (Windows)

Once  is downloaded on your system, you have to download Forensim and its dependencies.

### 1.2.2 Install the Forensim package

To make the forensim package fully functional in R you need some additional packages. These packages are: gdata, gtools, combinat, MASS, mvtnorm, genetics, tcltk, tcltk2, and tkrplot.

Forensim and its dependencies can also be found on the CRAN website <http://www.r-project.org/>. In the left menu under Software click the link Packages.

Click the link with the appropriate file. If you use windows it is the one next to Windows binary, for the Forensim package, it is the forensim\_2.0.zip file. Save the file in to your working folder. **Do not unzip the file.** Do this for all the following packages.

1. gdata
2. gtools
3. combinat
4. MASS
5. mvtnorm
6. genetics
7. tcltk
8. tcltk2
9. tkrplot.

Important note: if you cannot download the packages from CRAN website, it is probably because the mirror site you chose is not up to date. You can either try another mirror, or choose the Netherlands mirror, all packages needed are up to date on that mirror.

### 1.2.3 Install and load packages in R

All downloaded packages now need to be activated in R. Follow these steps:

- Open R
- Install packages using the R function `install.packages()`: for example NA

```
> install.packages('forensim_2.0.zip', repos=NULL)
```

Do this for every downloaded package. Change the information within the quotation marks according to each package. Load the package `forensim` to your current R session using the function `library()`:

```
> library(forensim)
```

```
### forensim 2.0 is loaded ###
```

The `forensim` package is now ready to be used! Note! Every time R is closed and opened again a new session starts and the `forensim` package needs to be loaded again, using the command “`library(forensim)`”.

**Tip for windows users** Download all the zip files in the same folder, then click on the Packages tab: install packages from zip files. It is possible to select all the packages at once, and install them at the same time (see figure 2).

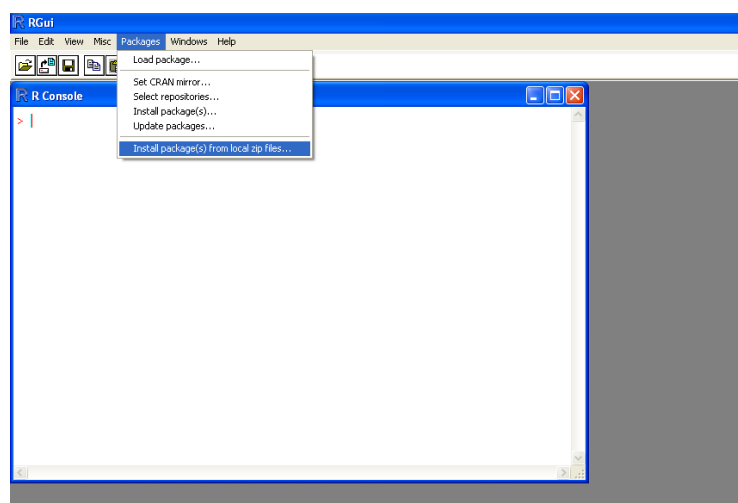


Figure 2: Package download in (Windows system).

The present document serves to introduce a particular functionality of the `Forensim` package, the `LRmix` module.

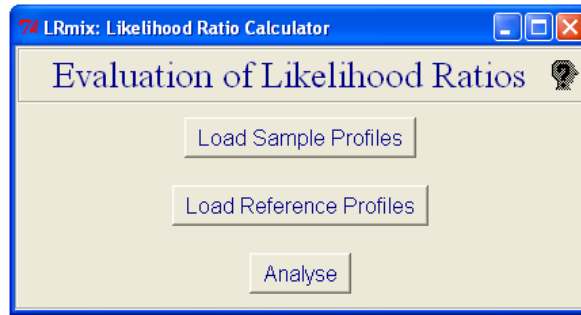


Figure 3: LRmix main graphical user interface

## 2 The LRmix module

Forensim implements a number of statistical methods (see Table) that can be used in the statistical interpretation of evidentiary DNA samples. These methods are documented in the manual of the Forensim package as well as in Haned (2010).

The LRmix module implements a model for the qualitative evaluation of DNA samples. It is a direct implementation of the model described in Curran et al. (2005). The LRmix module allows the calculation of likelihood ratios for different replicates, with any number of contributors, and in case dropout and drop-ins occur. Population substructure is also accounted for using the classical  $\theta$  correction (Balding and Nichols, 1994).

### 2.1 Getting started

The first step is to launch R. To do some simply click on the blue R icon. This should open an R session as shown in Figure 1.

The LRmix module is programmed into the R language, and its graphical user interface is programmed in Tcl/Tk. To launch the module, you have to simply type the following R commands into the R session:

Load the package forensim to your current R session using the function `library()`:

```
> library(forensim)
```

```
### forensim 2.0 is loaded ###
```

This command loads the library into your R session, which will enable you to use all the functions available in Forensim. The LRmix module is launched by the `LRmixTK()` command<sup>1</sup>.

```
> LRmixTK()
```

This launches a window that is the main interface to the LRmix module (figure 3): To be able to use the module you have to make sure that your R session is open, but you can minimize the R windows, and continue using the LRmix interface independently.

<sup>1</sup>TK stands for Tcl/Tk, the programming language used for the graphical user interface

The module has three buttons that correspond to three steps: first, load the sample profiles, second, load the reference profiles, and third, interpret the evidence using likelihood ratios.

## 2.2 Load sample Profiles

Pressing this button launches a window that allows you to select the files that contain the profiles of the evidence.



Figure 4: LRmix file upload window for the evidence profile.

The input files can either be text or CSV files. The typically obtained by exporting your data using Genotyping software as text file table. Table 1 gives an example of such file. The names of the replicates must be indicated using the SampleName column. The Marker column indicates the names of the markers. In this example Table 1, the user chose to use the data for the first six alleles. In practice, any number of alleles can be provided to the software. Empty or NA columns will be ignored by LRmix.

Once the file is chosen, the program allows you to see the profiles, and to eventually select the loci as well as the replicates to be analysed. Note that for the purpose of the course, only four replicates can be analysed simultaneously.

SampleName	Marker	Allele1	Allele2	Allele3	Allele4	Allele5	Allele6
R1	AMEL	X	Y				
R1	D3S1358	14	16				
R1	VWA	15	16	19.00			
R1	D16S539	11	13	14.00			
R1	D2S1338	20	23	24.00	25.00		
R1	D8S1179	11	12	13.00	15.00		
R1	D21S11	28	31				
R1	D18S51	13					
R1	D19S433	12	14	15.20	17.20		
R1	TH01	6	8	9.00	9.30		
R1	FGA	22					

Table 1: Required format for the input file for the evidence profile(s).

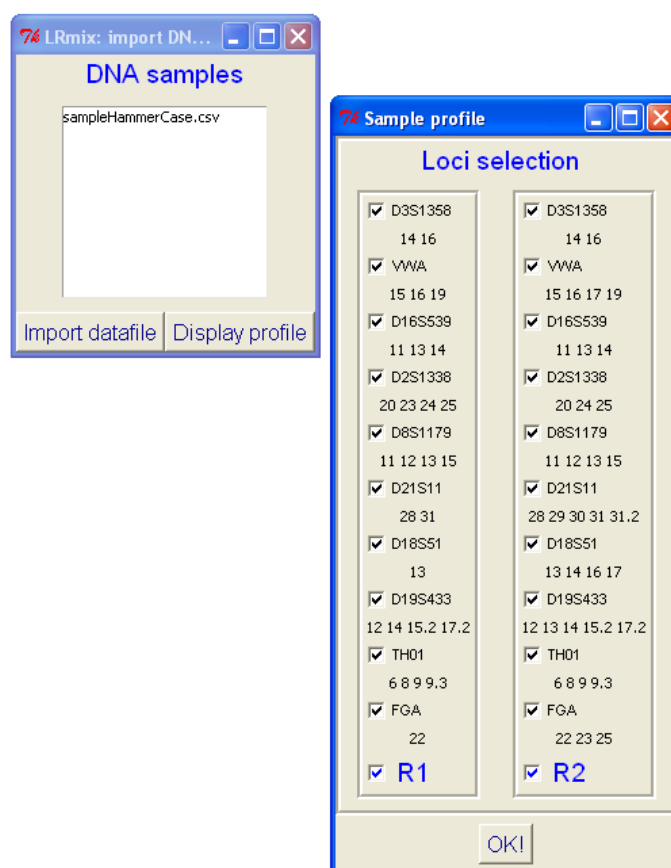


Figure 5: DNA profiles from the Hammer case.

Once your choice is made, simply press OK!, this will close the window. The program has recorded your preferences.

## 2.3 Load reference profiles

The next step now is to load the reference profiles, namely the suspect and the victim. Simply press OK when you finish uploading.



Figure 6: Uploading the reference DNA profiles from the Hammer case.

The selected files should be in the same format as the files used for the sample file (see Table 2). Obviously, we only expect the reference profiles to have two alleles. Any number of suspects and victims can be uploaded into the program.

SampleName	Marker	Allele1	Allele2
suspect	AMEL	X	Y
suspect	D3S1358	14	16
suspect	VWA	15	19
suspect	D16S539	11	14
suspect	D2S1338	24	25
suspect	D8S1179	12	13
suspect	D21S11	28	31
suspect	D18S51	14	17
suspect	D19S433	15.2	17.2
suspect	TH01	9	9.3
suspect	FGA	22	23

Table 2: Required format for the input file for the reference profile(s).



## 2.4 Analysis

The analysis button launches a window where you have to specify the model parameters.

Figure 7: Analysing the DNA profiles from the Hammer case.

By default the model selects the suspect and the victim (if provided) as the contributors under Hp, and the victims as the contributors under Hd. The suspect is automatically non-contributor under Hd.

The unknown numbers of contributors must also be specified under each hypothesis. Finally the probabilities of dropout and drop-in must be specified, default values are 0.1 and 0.01 respectively. The theta correction is set to zero by default. Different values of dropout probabilities are applied to homozygotes and heterozygotes. We denote  $D$  the probability of dropout for heterozygotes and  $D_2$  the probability of dropout for homozygotes. Following Balding and Buckleton (2009):  $D_2 = \alpha D^2$ . In LRmix,  $\alpha = \frac{1}{2}$ . The OK button launches the computations, and the results are displayed in a separate window. The LR is given per locus and overall loci by multiplying the per-locus values.

**Allele frequencies** The allele frequencies can be chosen among two datasets:

- SGM+ allele frequencies for the US Caucasian population (Butler et al., 2003).
- SGM+ allele frequencies for the Norwegian population (Andreassen et al., 2007).
- NGM allele frequencies (Budowle et al., 2011).

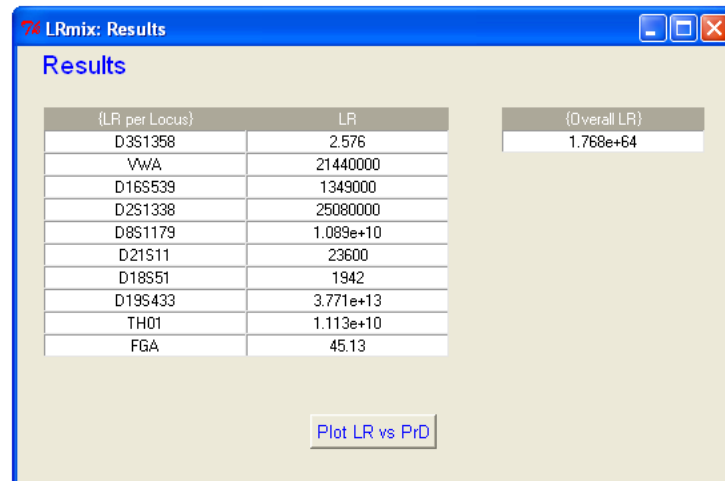


Figure 8: Likelihood ratios obtained for the Hammer case.

Note that it is also possible to plot the LR for varying values of the probability of dropout. This functionality will be further explored during the practical session.

### 3 Application

Two cases are explored during the practical session, they both involve the analysis of mixed DNA stains. The data files containing the DNA profiles are available on Forensim website, at the ISFG 2011 tab. Three CSV files are provided for each case:

- Sample: csv file containing the tabulated profiles of two PCR amplifications of the Norwegian case crime scene samples. The sample is analysed with the SGM+ kit.
- Suspect: csv file containing the tabulated profile of the suspect.
- Victim: csv file containing the tabulated profiles of the victim(s).

The tabulated profiles for the two cases are given in the folders Case 1 and Case 2, available as zipped files on Forensim website. Further information about each case will be given during the course.

## References

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