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Introduction to the LRmix program of the Forensim R package

Oskar Hansson

(slides adapted from Hinda Haned and Peter Gill,
Advanced DNA Interpretation workshop ISFG 2013, Melbourne)

For news updates subscribe to
forensimnews@gmail.com

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Install the R software

www.cran.r-project.org

The Comprehensive R Archive Network

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Download and Install R

Precompiled binary distributions of the base system and contributed packages, **Windows and Mac** users most likely wan

- Download R for Linux
- Download R for MacOS X
- Download R for Windows

R is part of many Linux distributions, you should check with your Linux package management system in addition to the li

Source Code for all Platforms

Windows and Mac users most likely want to download the precompiled binaries listed in the upper box, not the source co compiled before you can use them. If you do not know what this means, you probably do not want to do it!

- The latest release (2012-06-22, Roasted Marshmallows): R-2.15.1.tar.gz, read what's new in the latest version.
- Sources of R alpha and beta releases (daily snapshots, created only in time periods before a planned release).
- Daily snapshots of current patched and development versions are available here. Please read about new features an corresponding feature requests or bug reports.
- Source code of older versions of R is available here.
- Contributed extension packages

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Subdirectories:

base	Binaries for base distribution (managed by Duncan Murdoch). This is what you want to **install R for the first time**.
contrib	Binaries of contributed packages (managed by Uwe Ligges). There is also information on third party software available for CRAN services and corresponding environment and make variables.
Rtools	Tools to build R and R packages (managed by Duncan Murdoch). This is what you want to build your own packages on Windows, c itself.

Please do not submit binaries to CRAN. Package developers might want to contact Duncan Murdoch or Uwe Ligges directly in case of questions / suggestions rela

You may also want to read the R FAQ and R for Windows FAQ.

Note: CRAN does some checks on these binaries for viruses, but cannot give guarantees. Use the normal precautions with downloaded executables.

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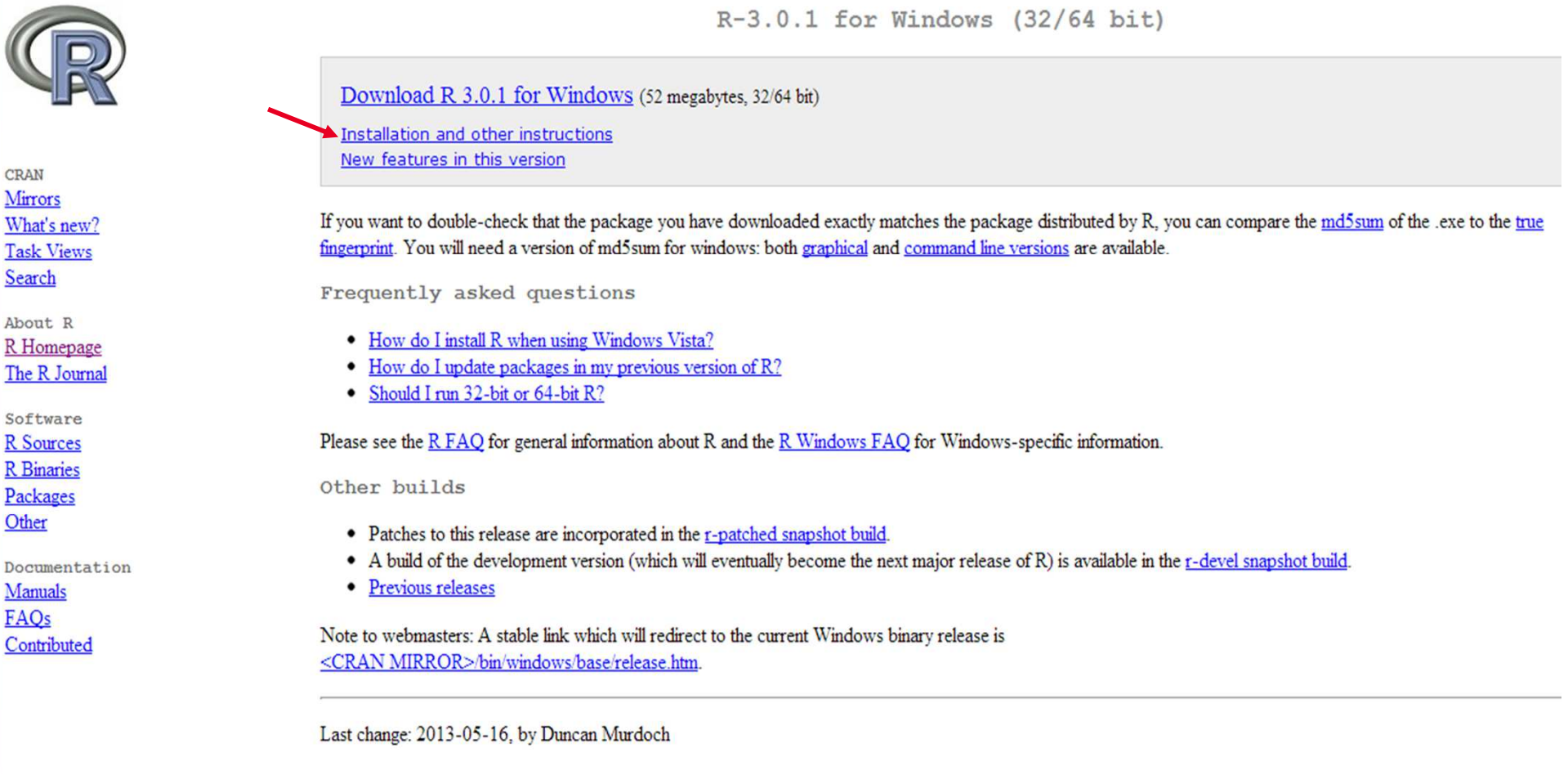
Install the R software



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R-3.0.1 for Windows (32/64 bit)

Download R 3.0.1 for Windows (52 megabytes, 32/64 bit)

Installation and other instructions
New features in this version

If you want to double-check that the package you have downloaded exactly matches the package distributed by R, you can compare the md5sum of the .exe to the true fingerprint. You will need a version of md5sum for windows: both graphical and command line versions are available.

Frequently asked questions

- How do I install R when using Windows Vista?
- How do I update packages in my previous version of R?
- Should I run 32-bit or 64-bit R?

Please see the R FAQ for general information about R and the R Windows FAQ for Windows-specific information.

Other builds

- Patches to this release are incorporated in the r-patched snapshot build.
- A build of the development version (which will eventually become the next major release of R) is available in the r-devel snapshot build.
- Previous releases

Note to webmasters: A stable link which will redirect to the current Windows binary release is <CRAN MIRROR>/bin/windows/base/release.htm.

Last change: 2013-05-16, by Duncan Murdoch

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Install the R software

- An executable file will be downloaded.
- R.3.0.1.exe (or newer version)
- Simply click and follow the instructions!

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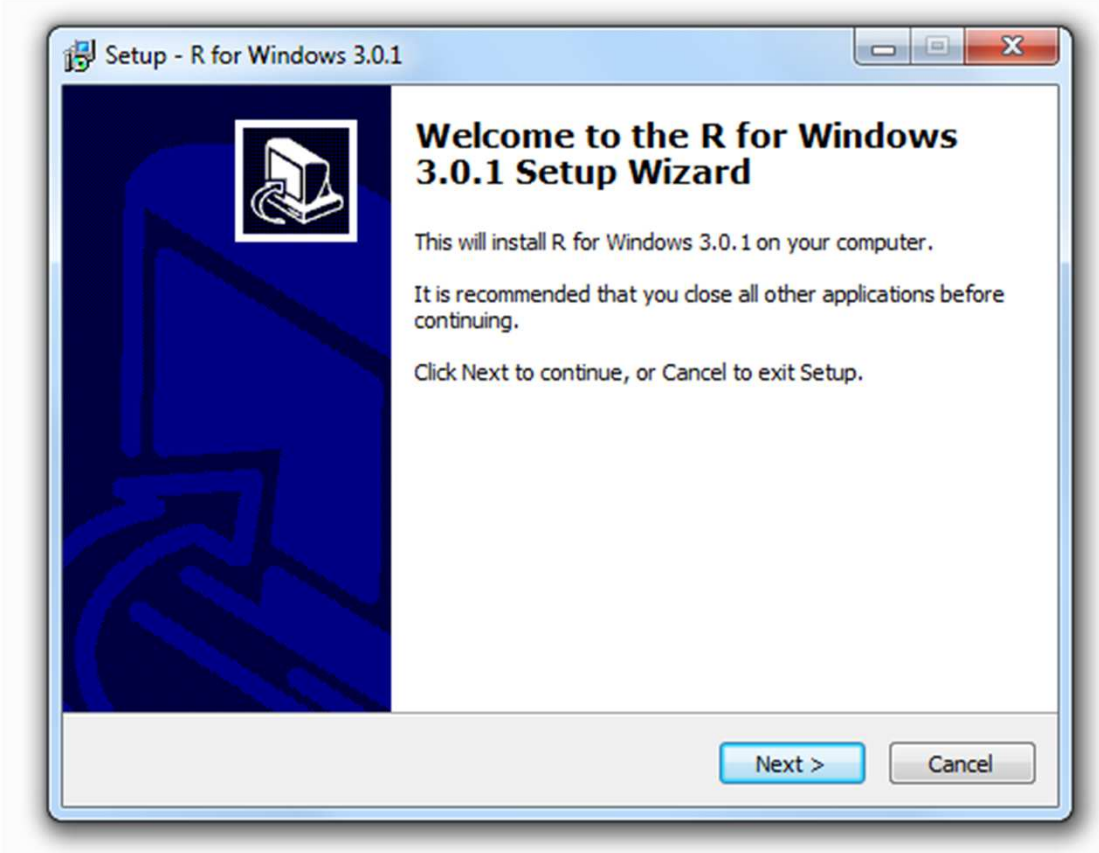
Install the R software



Press ‘next’ until...

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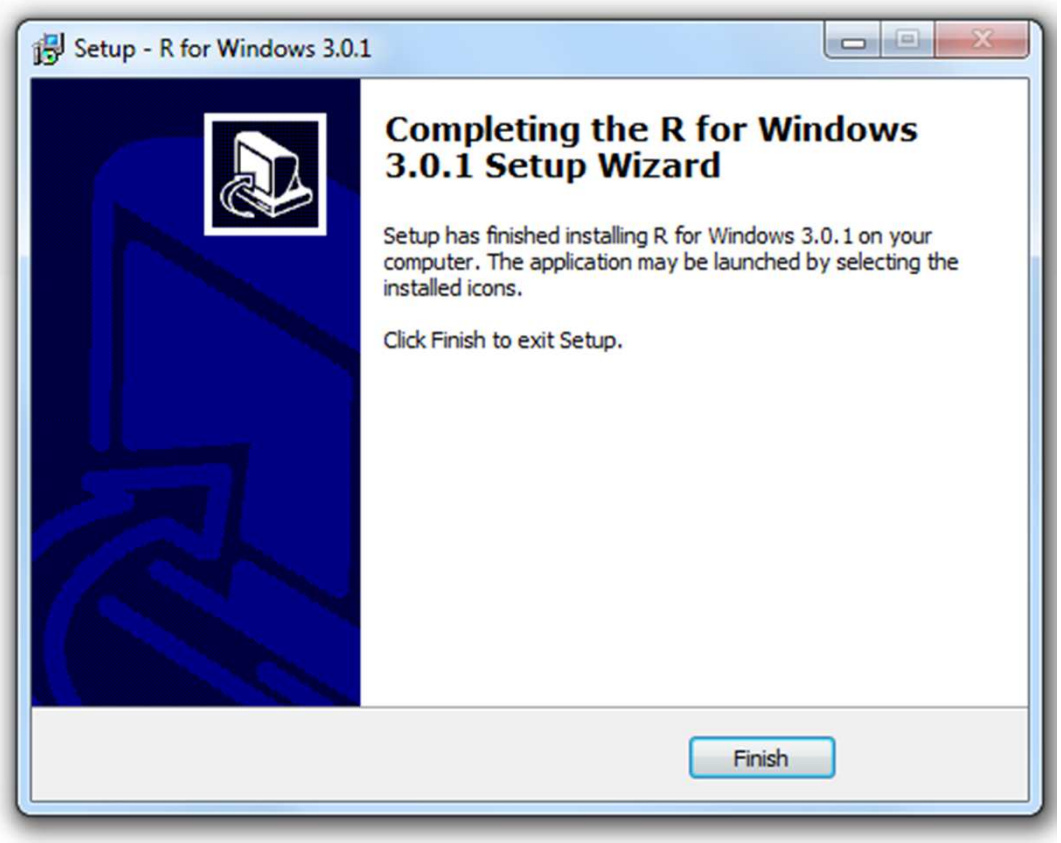
Install the R software



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Prepare your working folder (make sure this is set up before the lecture)

- You have been sent some data-sets in folders – place these into a folder on your computer
- Place a short cut to R in the same folder (you can drag the R icon from your desktop into your folder)

NB! in screen shots on the following slides “your folder” is named “Melbourne” and the sub-folders might differ from the ones you have. Also note that your operating system might have a different appearance.

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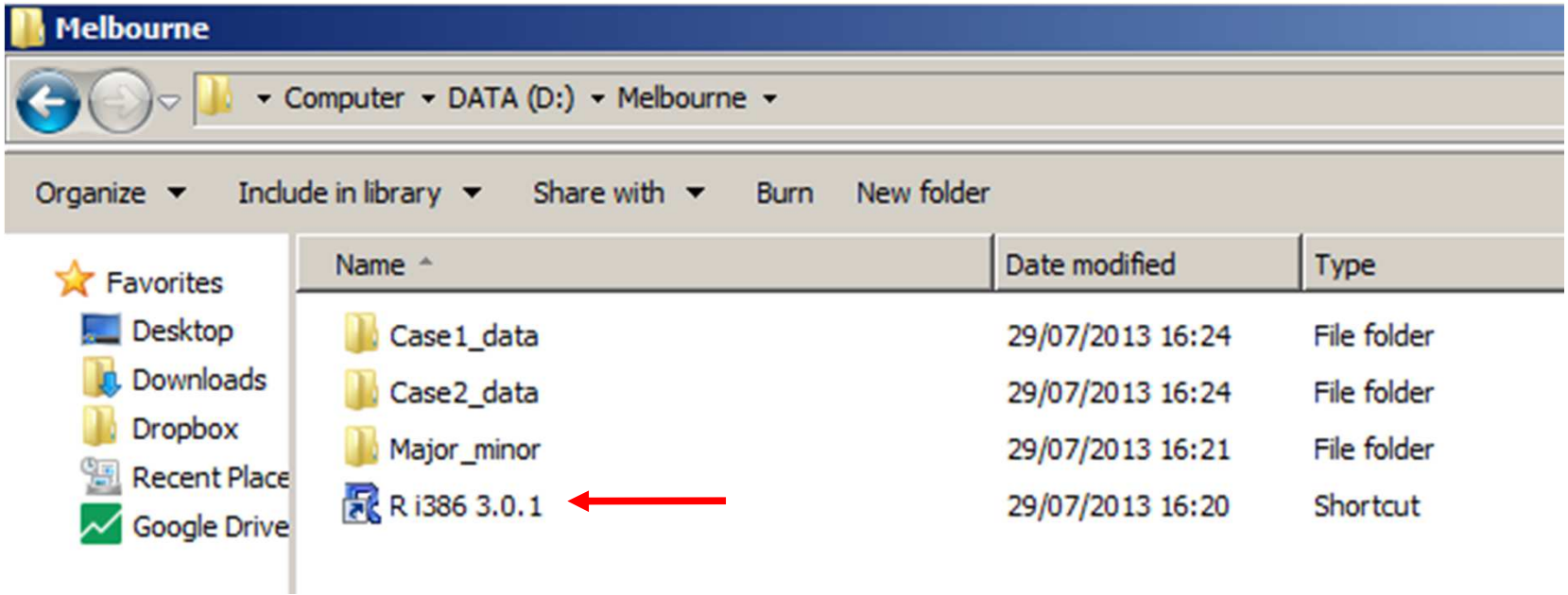
You are ready to launch R

Double-Click blue icon.



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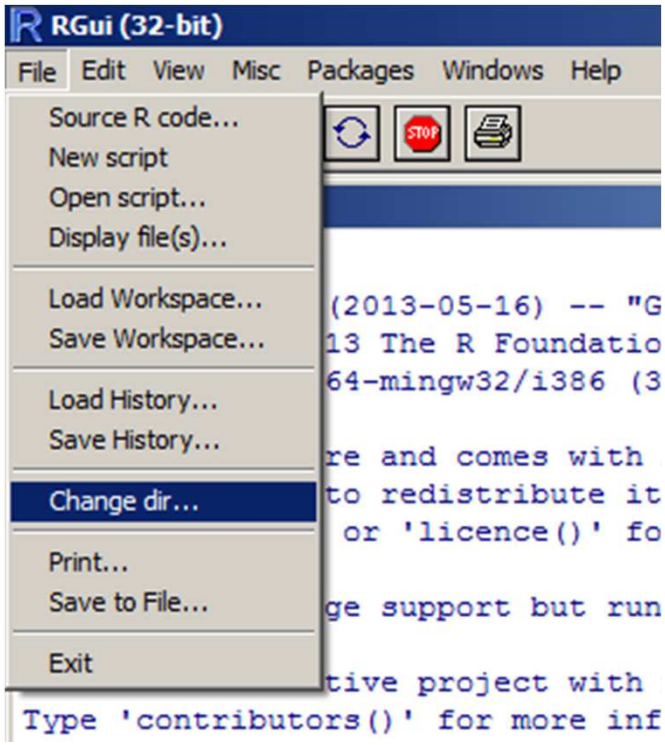
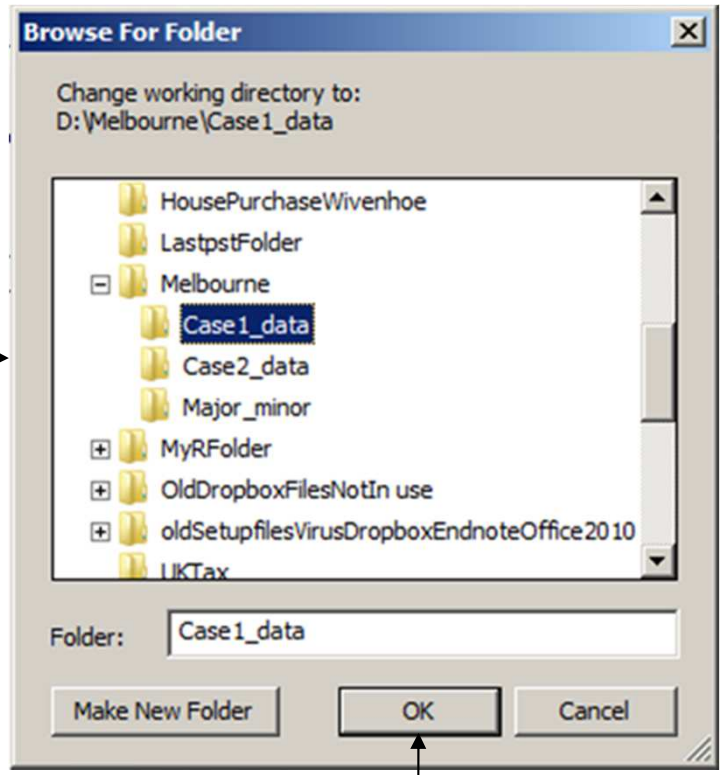
Set directory to your folder





Press OK to set directory

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Install the Forensim package

- Option 1: install the package directly from the R environment (Internet connection) - please follow this option now.
  - Also download LRmix tutorial from:

    http://forensim.r-forge.r-project.org/misc/LRmix.pdf

- Option 2: Install the package manually (no Internet connection)
  - ☛ Refer to LRmix tutorial online

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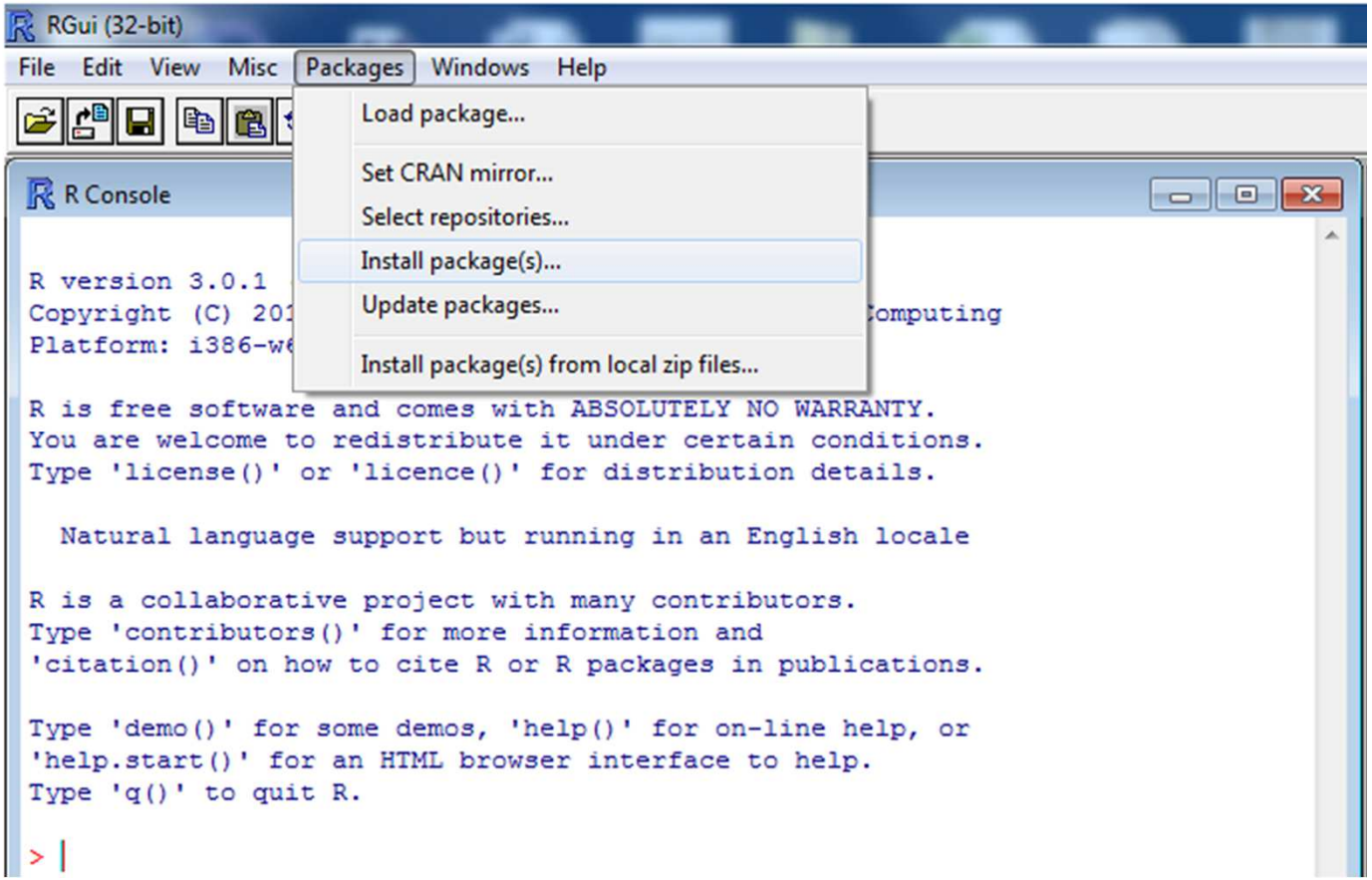
Install the Forensim package



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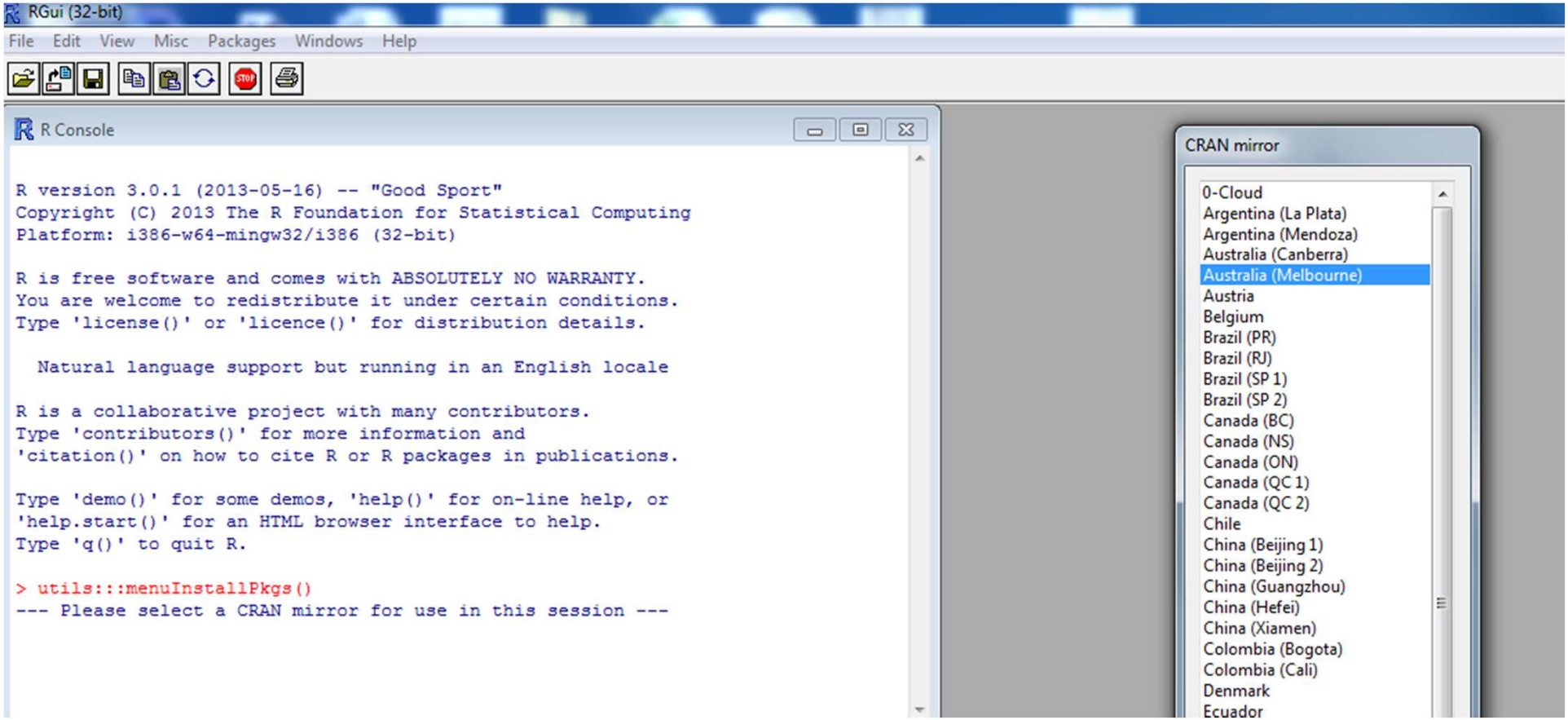
Install the Forensim package

Choose a mirror that is geographically close to you



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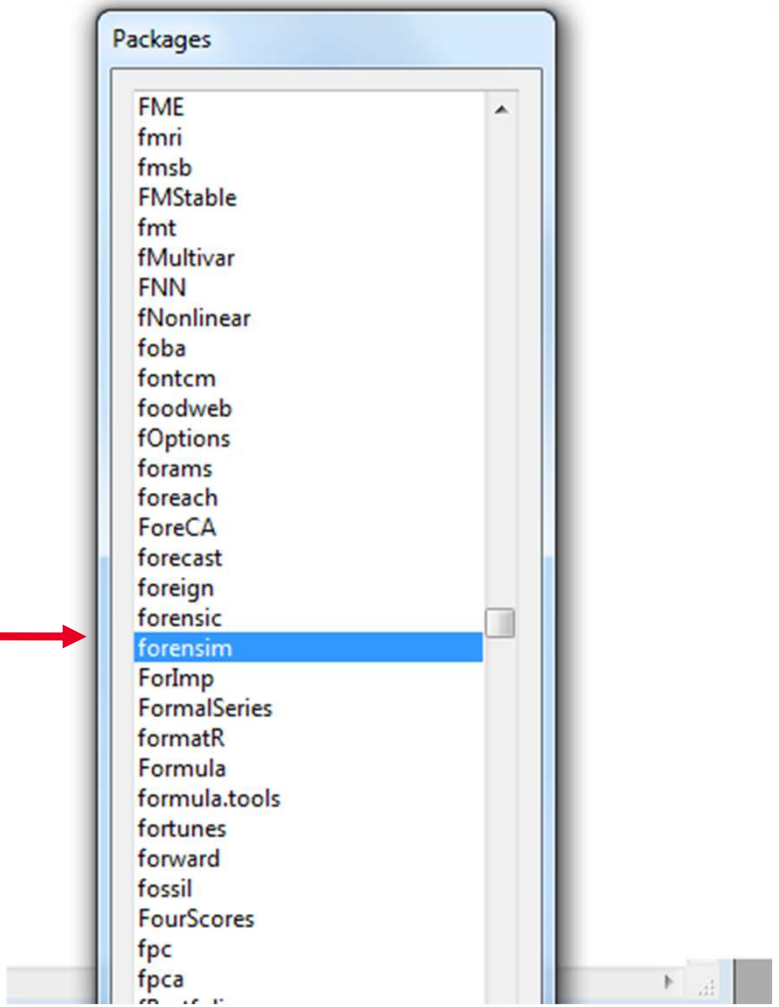
Choose package forensim



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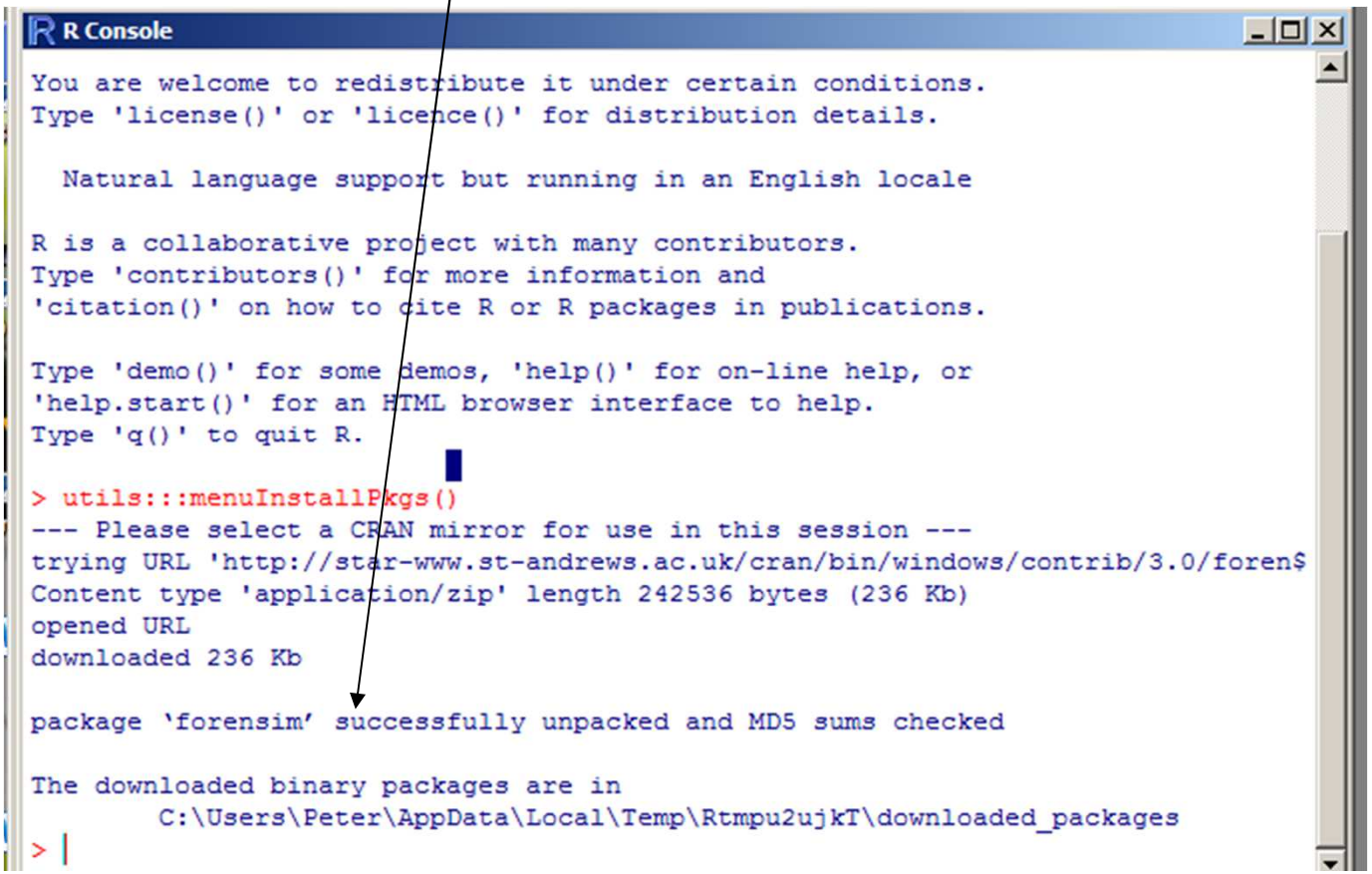
Your screen should look something like this Make sure you have a message:" 'forensim' successfully unpacked"



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- Please try to get this far, and make sure you bring a laptop with the R program and files preloaded as described in the previous slides.

- This will save us a lot of time if you can do this.

- If you have a problem up to here, please contact me for advice: oskar.hansson@fhi.no

- For those who are interested, you may wish to attempt to start an analysis of the first case

- Continue to the next slide to do this

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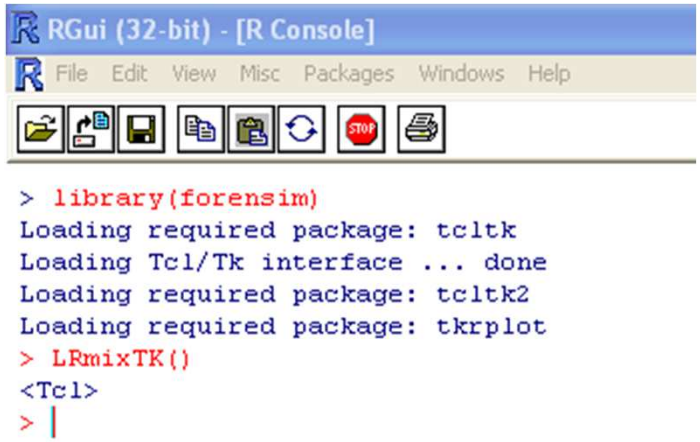
Start LRmix

Type “library(forensim)” (without quotes) in the R console and hit Enter. This loads the Forensim package.



Then type “LRmixTK()” to start the LRmix graphical user interface.

NB! Commands in R are case sensitive.

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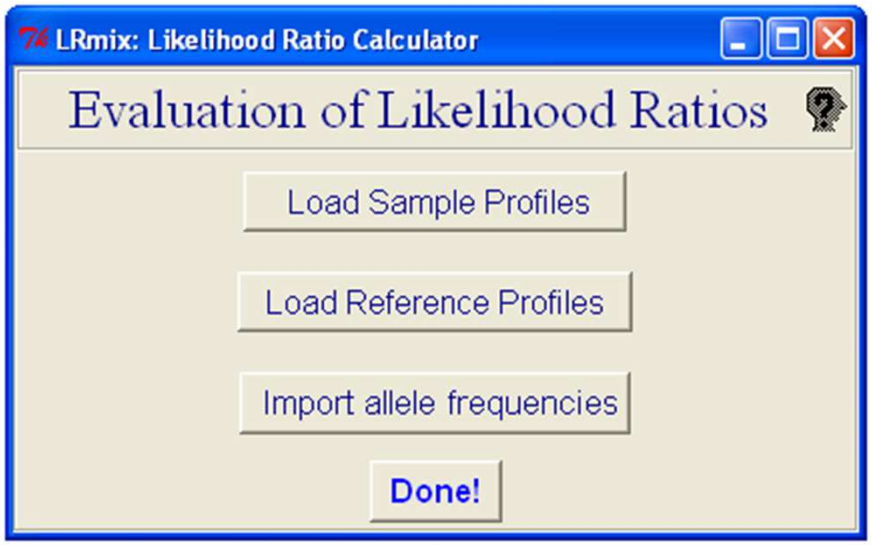
The main LRmix interface



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Input files in Lrmix (NB! The data files are already in your folder)

Type 1: CSV files, they are comma separated files (‘,’), and the decimal separator is the dot (‘.’)

Type 2: tab separated files, they are tab separated (‘\t’, e.g. Excel), and the dot(‘.’) is the decimal separator

Never use spaces in your column-names, or in the sample-names (epg, or references)

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CSV file example

```
SampleName,Marker,Allele1,Allele2
Suspect1,AMEL,X,Y
Suspect1,D3S1358,16,17
Suspect1,VWA,16,18
Suspect1,D16S539,12,13
Suspect1,D2S1338,19,20
Suspect1,D8S1179,9,13
Suspect1,D21S11,28,32
Suspect1,D18S51,12,15
Suspect1,D19S433,12,16
Suspect1,TH01,6,9.3
Suspect1,FGA,19,21
Suspect2,AMEL,X,Y
Suspect2,D3S1358,15,17
Suspect2,VWA,18,19
Suspect2,D16S539,12,12
Suspect2,D2S1338,17,18
Suspect2,D8S1179,13,13
Suspect2,D21S11,30,30
Suspect2,D18S51,12,20
Suspect2,D19S433,12,15
Suspect2,TH01,6,9.3
Suspect2,FGA,20,21
```

CSV file opened in a raw 'non-destructive' text editing program like Notepad or Notepad++

	A	B	C	D
1	SampleName	Marker	Allele1	Allele2
2	Suspect1	AMEL	X	Y
3	Suspect1	D3S1358	16	17
4	Suspect1	VWA	16	18
5	Suspect1	D16S539	12	13
6	Suspect1	D2S1338	19	20
7	Suspect1	D8S1179	9	13
8	Suspect1	D21S11	28	32
9	Suspect1	D18S51	12	15
10	Suspect1	D19S433	12	16
11	Suspect1	TH01	6	9.3
12	Suspect1	FGA	19	21
13	Suspect2	AMEL	X	Y
14	Suspect2	D3S1358	15	17
15	Suspect2	VWA	18	19
16	Suspect2	D16S539	12	12
17	Suspect2	D2S1338	17	18
18	Suspect2	D8S1179	13	13
19	Suspect2	D21S11	30	30
20	Suspect2	D18S51	12	20
21	Suspect2	D19S433	12	15
22	Suspect2	TH01	6	9.3
23	Suspect2	FGA	20	21

CSV file opened in a spread-sheet program like Microsoft Excel or Libre Office Calc. Depending on the settings you may have to use a function like "Text to columns" and separate by comma (,).

If editing in a spread-sheet program be careful to save the file as a CSV file.

NB! Beware in countries using comma as decimal separator.

S11	30	30
S51	12	20
S433	12	15
1	6	09.mar
	20	21

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A case example

- The crime-stain is from an epithelial swab taken from the female victim
- There are two suspects accused of sexual assault, S_1 and S_2 respectively; both deny the offence.
- This epg is classified as a low template of three or more individuals since there are multiple alleles per locus that fall within the criterion of the low template zone (between the LDT and the stochastic threshold (T)) – we expect that dropout may occur, but the profiles appear to be well represented.

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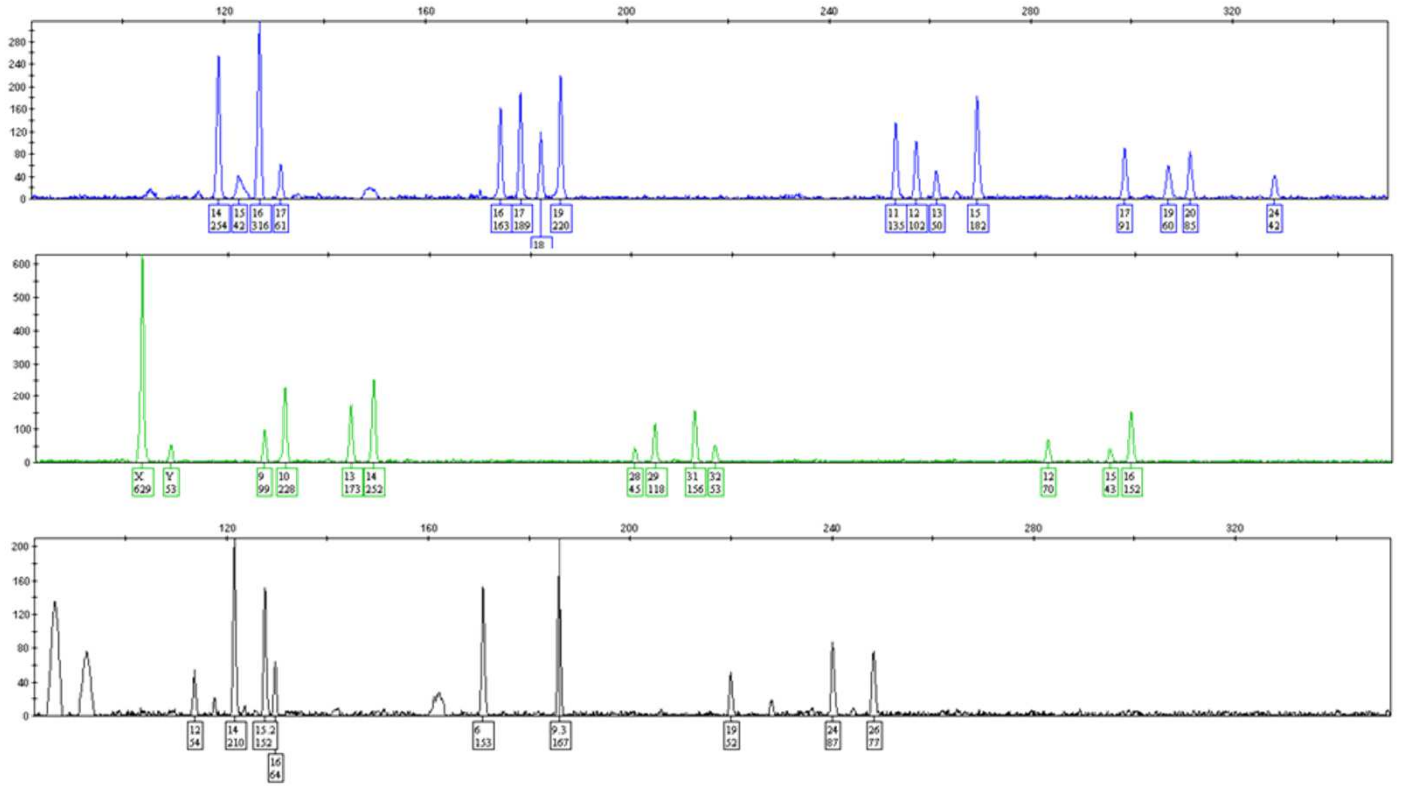
EPG



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List the alleles with informative formatting

	Crime-stain alleles								
Marker	Allele1	Allele2	Allele3	Allele4	S1	S1	S2	S2	Unique alleles
AMEL	X	Y			X	Y	X	Y	2
D3S1358	14	16	17	(15)	16	17	15	17	4
VWA	16	17	18	19	16	18	18	19	4
D16S539	11	12	13	15	12	13	12	12	4
D2S1338	17	19	20	(24)	19	20	17	18	4
D8S1179	9	10	13	14	9	13	13	13	4
D21S11	29	31	32		28	32	30	30	5
D18S51	12	16	(15)		12	15	12	20	4
D19S433	12	14	15.2	16	12	16	12	15	5
TH01	6	9.3			6	9.3	6	9.3	2
FGA	19	24	26		19	21	20	21	5

Key:
Alleles that are shared between victim and S_1 or S_2 (green background).
Alleles that are found in the crime stain and not observed in any known individual (blue background, not applicable in this case).
Alleles that are below the detection threshold but appear to be distinct (bracketed).
Alleles that are found in the crime stain that match a known individual under *Hd* (victim) (red typeface).

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Establish the minimum number of contributors for the 'preliminary' propositions

a) The swab is from a victim (*V*). There are two suspects (S_1,S_2) under *Hp*,

b) In this example, some loci have 5 unique alleles across sets hence there is a minimum of three individuals present under *Hp*.

c) A similar calculation can be made under *Hd* where the sets of genotypes formed by S_1,S_2 are not used, but in our rationale, it is convenient to anchor the minimum number of contributors on *Hp* and to assume equivalence (this is revisited later in the procedure).

d) Consequently, the preliminary propositions are formulated as *Hp=V,*S_1,S_2 and *Hd=V,U,U*

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LRmix analysis

- *Hp=V,S_1,S_2* and *Hd=V,U,U*

- The $\log_{10}(LR_{min})$= 5.66 is derived for a drop-out probability *Pr*(*D*)=0.16.

- Pr(D) value is in fact the 5 percentile calculated from an empirical distribution of the drop-out probability conditioned on the expected number of alleles observed relative to the genotype of the hypothesised contributors, the procedure is described by Haned et al (FISG 2012)

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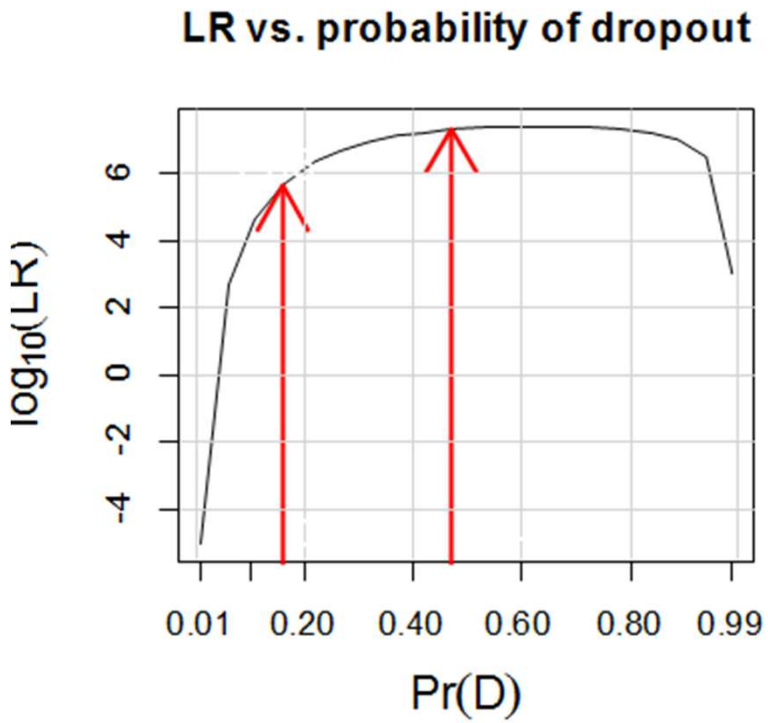
Sensitivity plot



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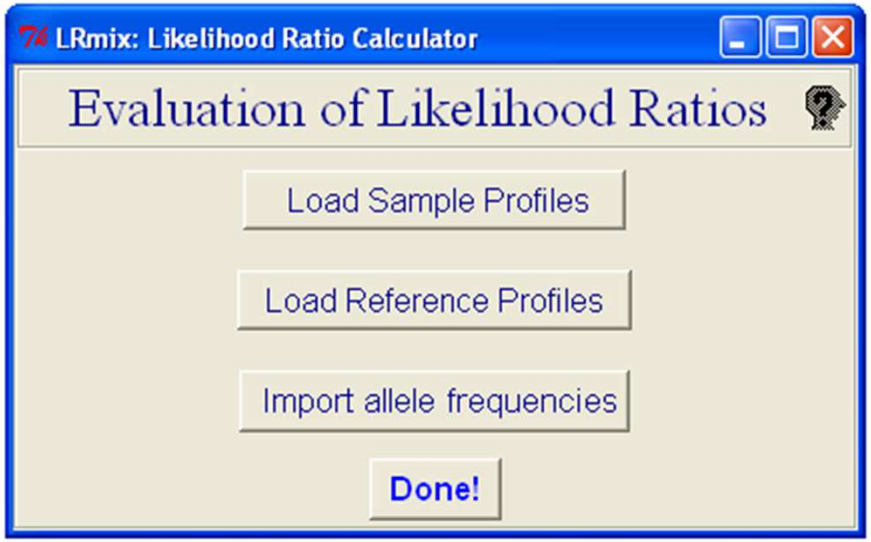
Main LRmix interface

Now we show how to:

(1)Load the crime-sample profile

(2)Load the references (suspect/victim)

(3)Load your allele frequencies



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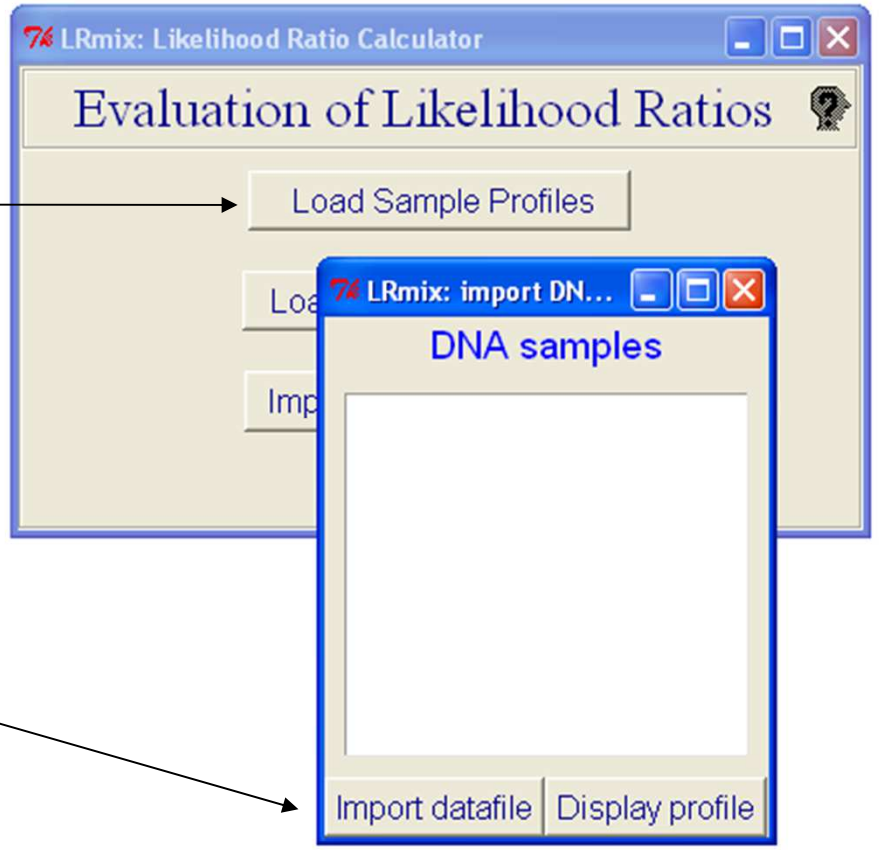
(1) Load the crime-sample profiles

Click "Load Sample Profiles"

Click "Import datafile"



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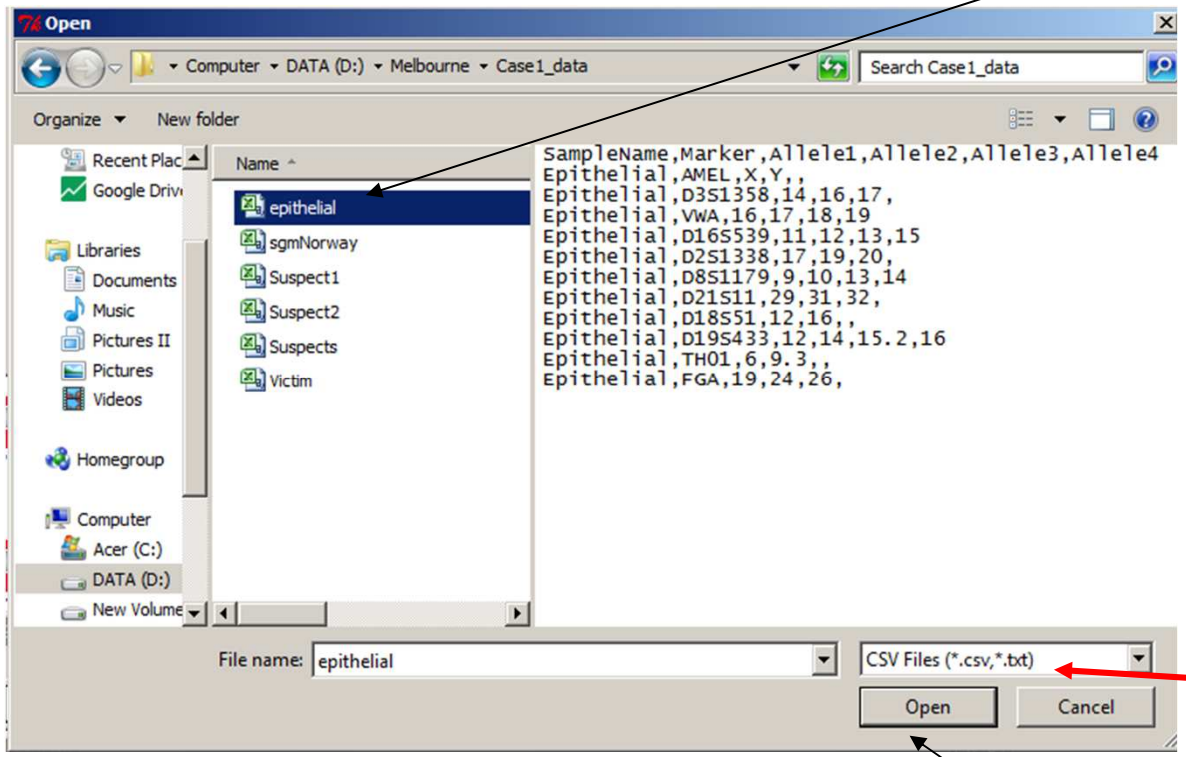
(1) Navigate to your folder and open the Case1_data folder
Select the crime-sample profile.

epithelial



Make sure this is set to CSV Files

Then click 'Open'

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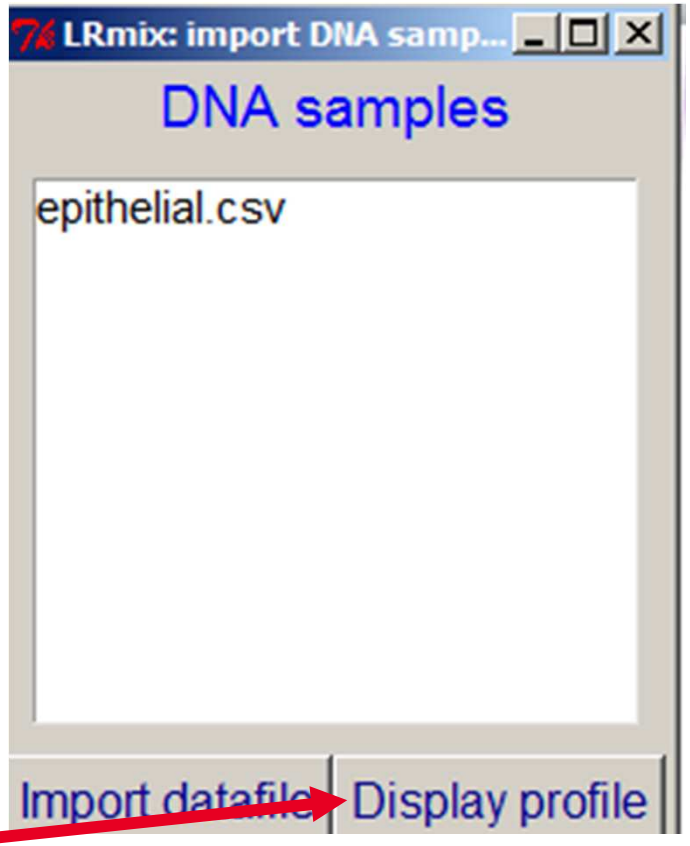
Display the crime-sample profile



Click ‘Display profile’,

To make sure the data is OK

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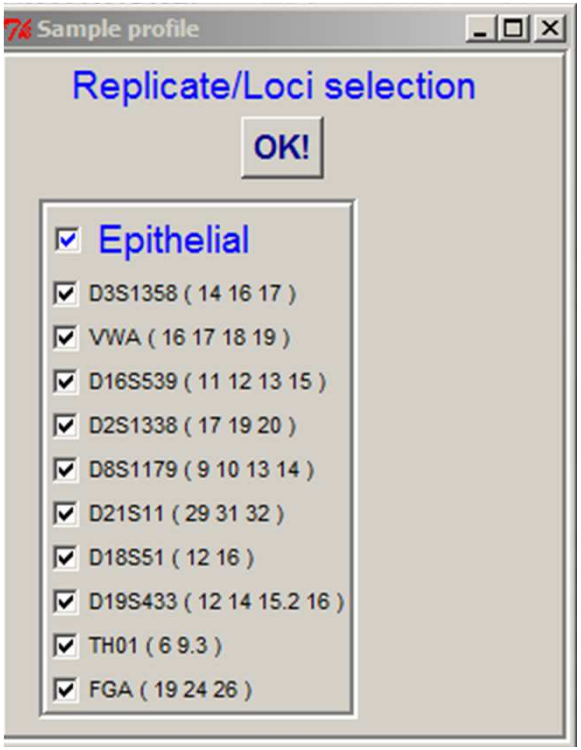
If everything looks good, press OK!

- You can select loci if you want
- But leave intact for this exercise



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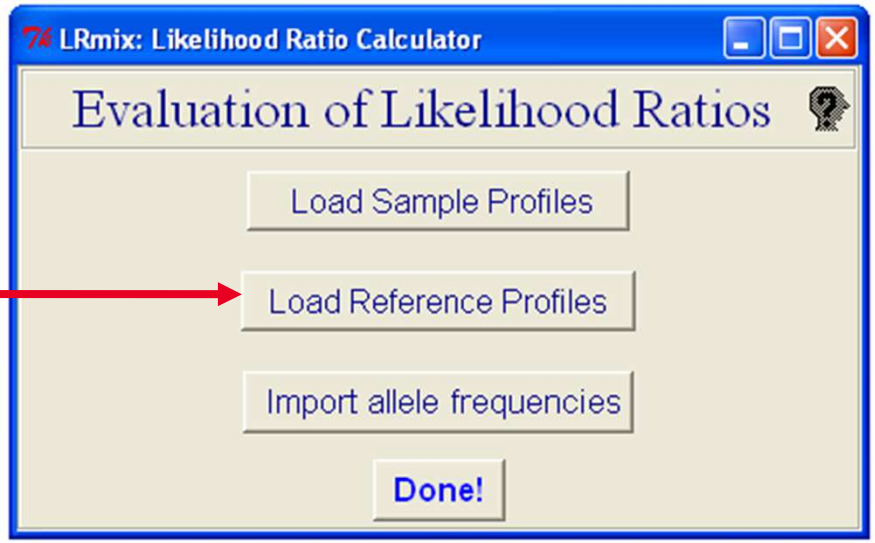
(2) Load reference profiles from your folder

suspects

victim



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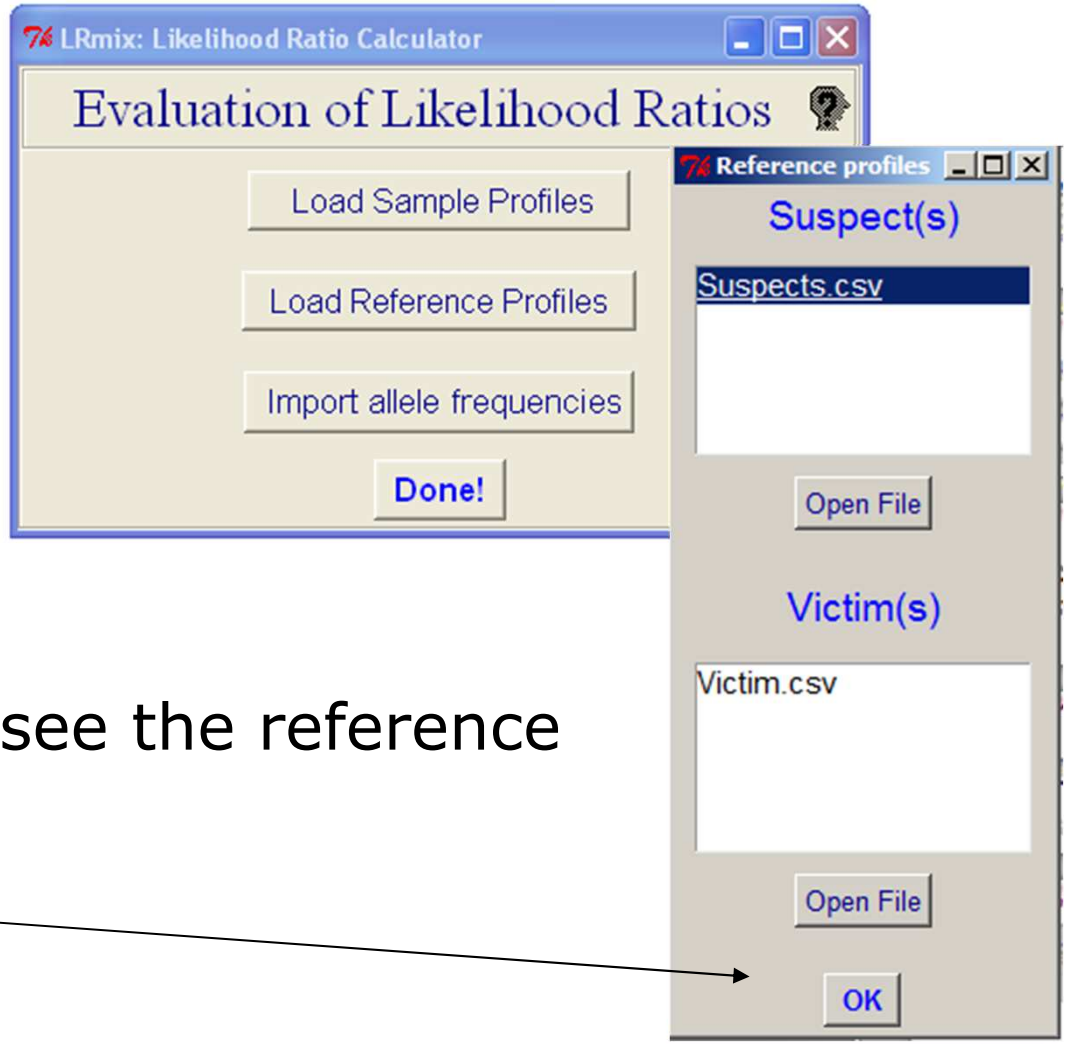
(2) Load reference profiles from your folder



You cannot see the reference profiles

Press OK

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- You cannot see the reference profiles
- The program will automatically select the loci you chose in step (1)
- If there are loci in the epg that are not given in the reference profile, the program will give an error message

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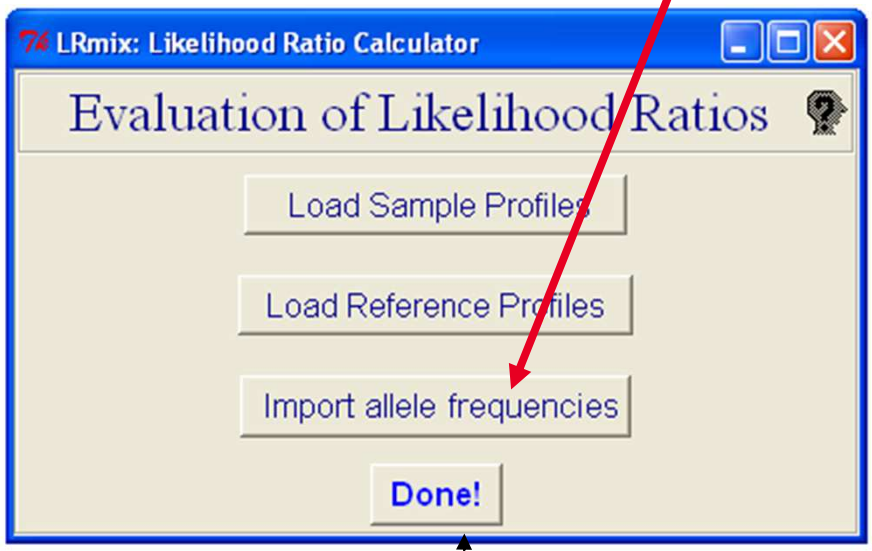
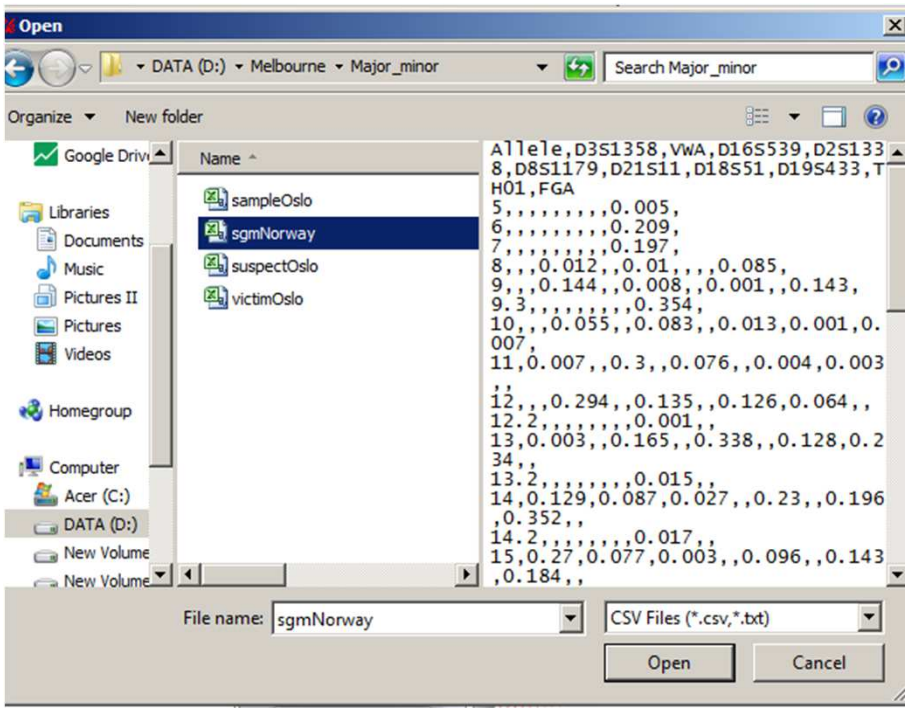
(3) Import the allele frequencies

sgmNorway





Once loaded, click ‘Done’

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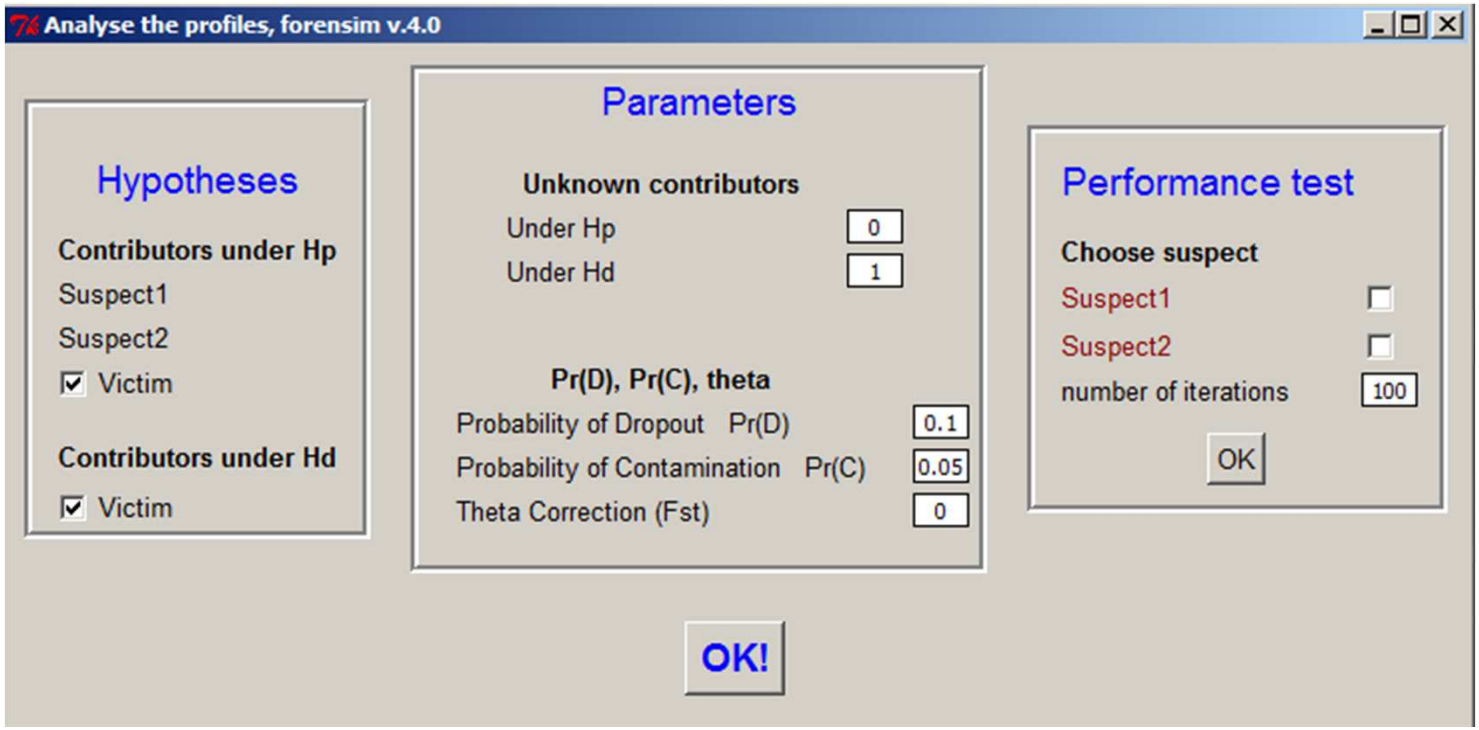
Now you should see this



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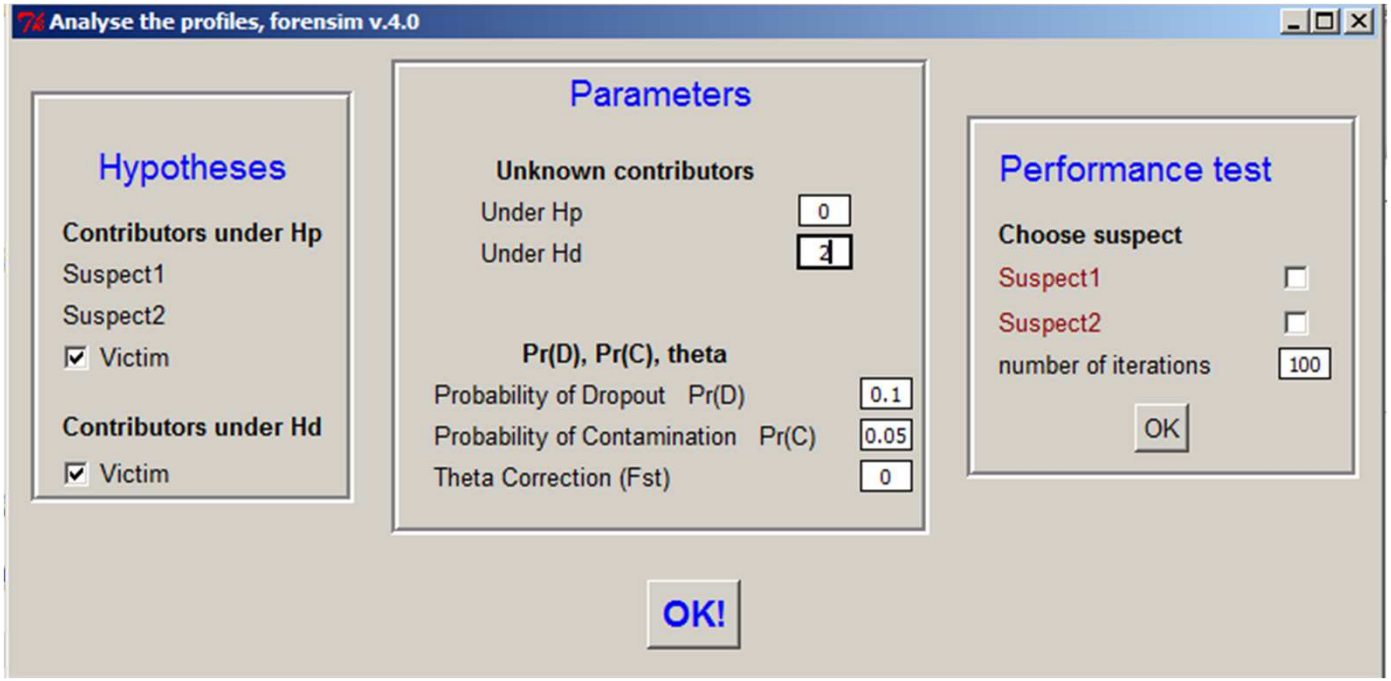
Alter the parameters

- 2 unknown contributors under Hd
- Click OK



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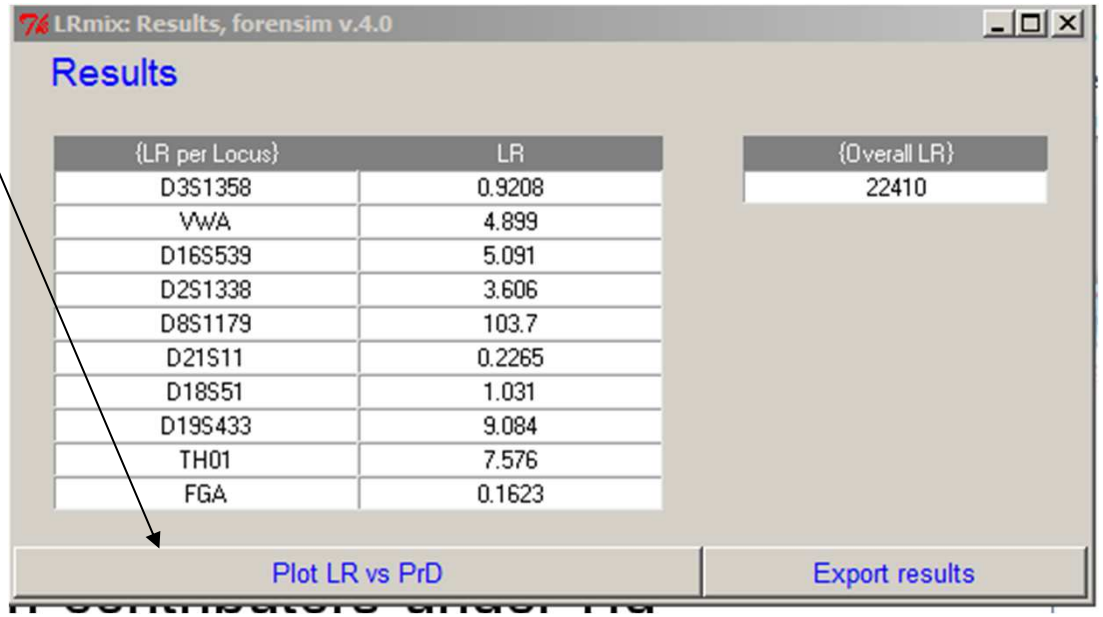
Results Table

- Carry out sensitivity analysis – click on button



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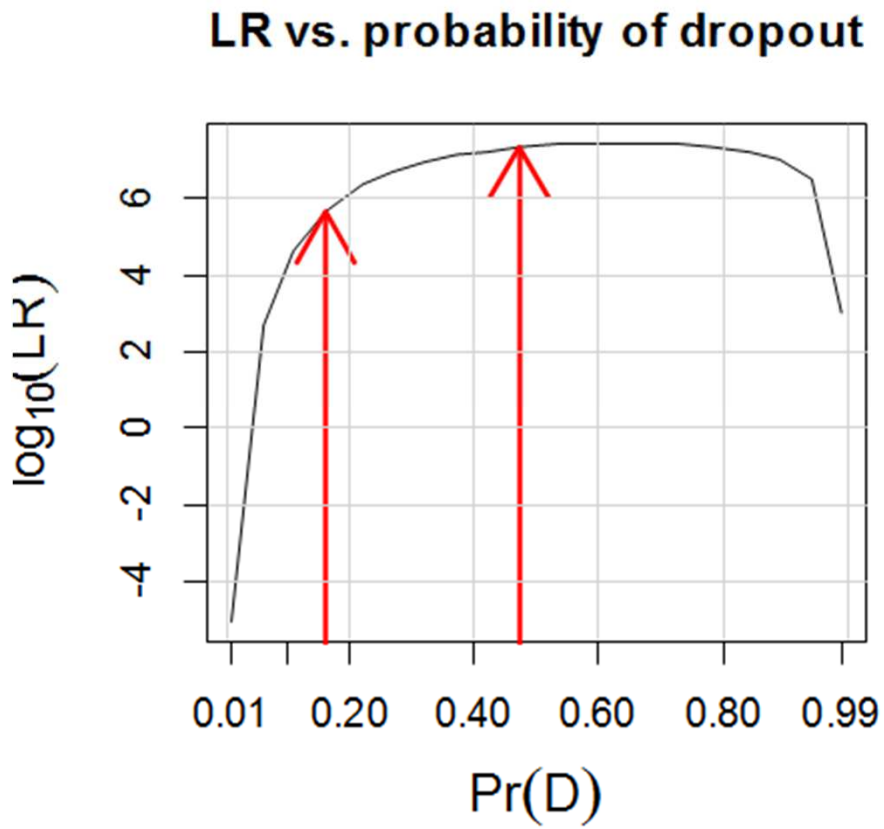
Result of sensitivity analysis



======== Drop-out ranges:
under Hp ========
5% percentile 0.22
95% percentile 0.42

======== Drop-out ranges:
under Hd ========
5% percentile 0.16
95% percentile 0.42

The red arrows delineate the reasonable range for Pr(D).
The LR≈10^6 .

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Case evaluation

- So far we have only done a partial evaluation
- Think about how you would further evaluate this case?
- Are the propositions reasonable?
- Would you like to evaluate any other propositions?
- What would a final statement look like?

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Recap (with further explanation)

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Why exploratory?

- The purpose is not to give a 'black-box' answer because there is no definitive answer
- All of the answers are conditional hence the function of the 'expert' is to explore the various possibilities, on behalf of the prosecution and defence.
- Some generalisations are possible
- The 'process' used to interpret complex DNA profiles is provided in this talk
- Consider a major/minor(s) contributors in the following epg. We could regard this as a typical LTDNA profile

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Step 1: examine the epg

- And Consider the case circumstances
- Is it a mixture?

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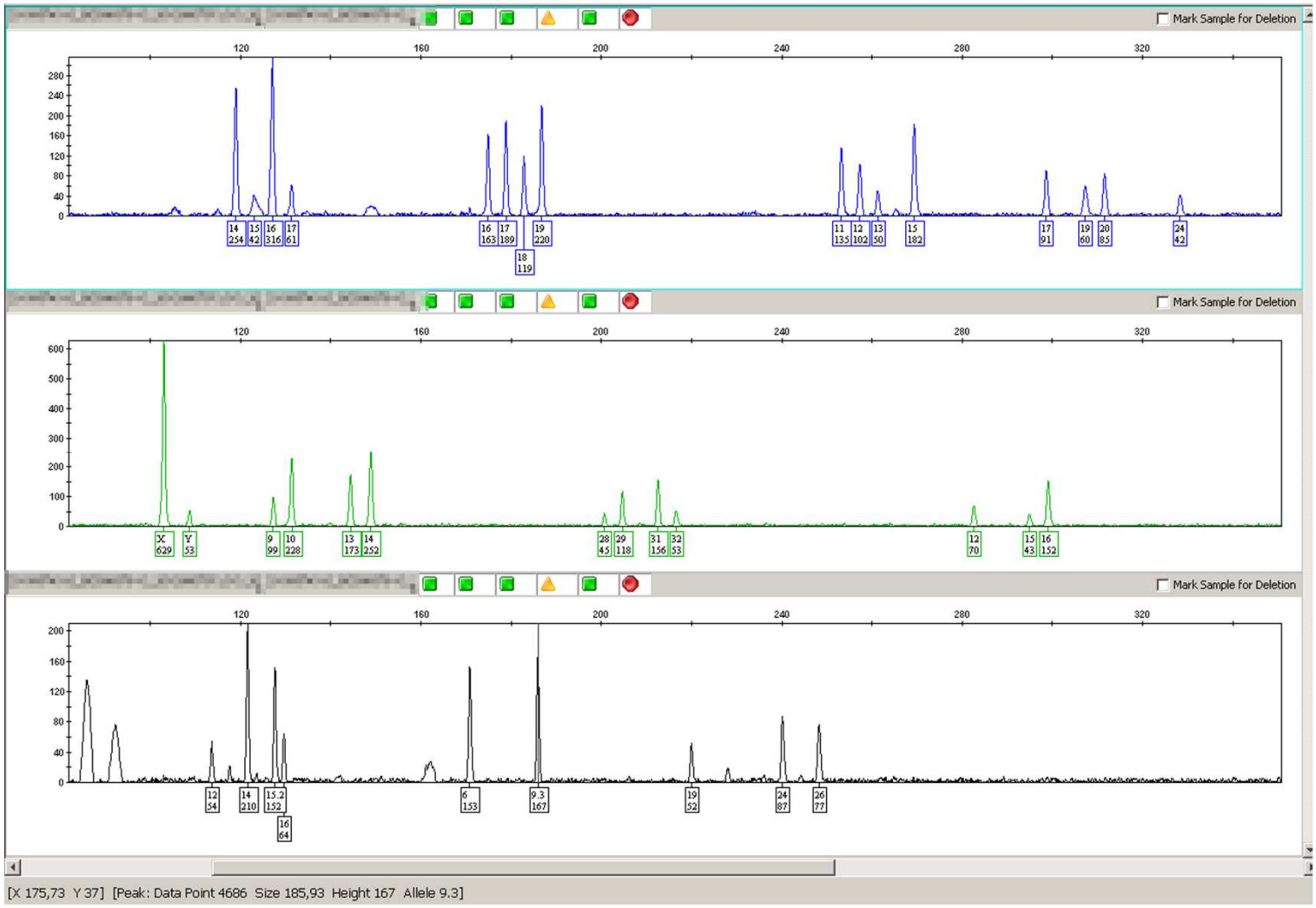
EPG

Case circumstances:

- Epithelial swab from female victim (V)
- Sexual assault with two suspects under Hp (S1, S2)



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Step 1: examine the epg

- What kind of mixture is it?
- Choose from following:
  - Major/minor?
  - Even?
- Do we expect drop-out?
  - (compare with logistic regression)

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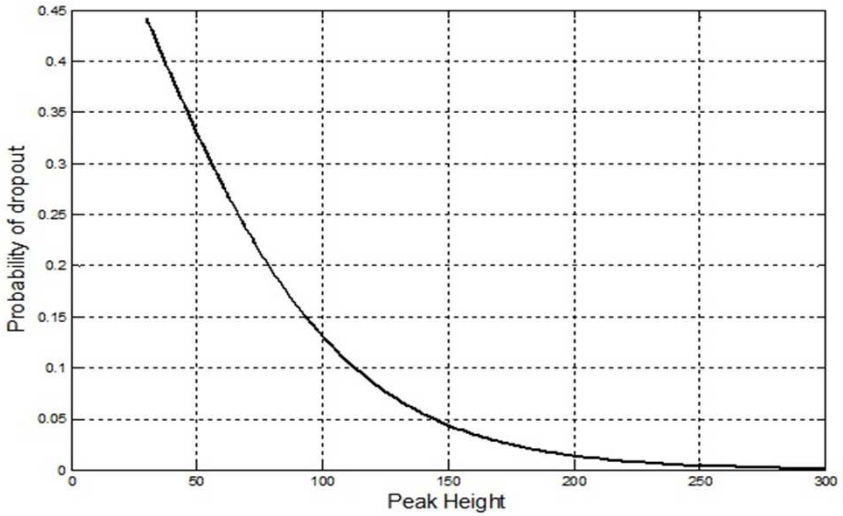
A typical low template profile showing Pr(D) range relative to peak height thresholds

Check the peak heights against logistic regression to work out if drop-out is expected



Stochastic T: Pr(D)≈0

LOD: Pr(D)≈0.35

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Change in philosophy

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- With the old methods we had to ‘filter’ alleles and there were many restrictions about the kind of analysis that could be undertaken
- The new method can evaluate profiles without filtering alleles and are not restricted by numbers of contributors etc.
- Consequently, we are able to devise simple rules that can be followed to produce an LR.
- The questions shift towards “what are the propositions that should be considered”
- The role of the reporting officer now becomes a facilitator of the court going discussion by following a logical process

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Step 2: Make a table of alleles in the case-stain and the known contributors

- A format is suggested in the next slide
- Note that the procedure here differs from the Clayton guidelines since we must condition the hypotheses using all the evidence under Hp – so this means that the reference samples are evaluated concurrently with the crime-stain
- However, all alleles are included so long as they are above the analytical threshold (AT or commonly LOD, limit of detection)

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Step 2: List the alleles with informative formatting

	Crime-stain alleles								
Marker	Allele1	Allele2	Allele3	Allele4	S1	S1	S2	S2	Unique alleles
AMEL	X	Y			X	Y	X	Y	2
D3S1358	14	16	17	(15)	16	17	15	17	4
VWA	16	17	18	19	16	18	18	19	4
D16S539	11	12	13	15	12	13	12	12	4
D2S1338	17	19	20	(24)	19	20	17	18	4
D8S1179	9	10	13	14	9	13	13	13	4
D21S11	29	31	32		28	32	30	30	5
D18S51	12	16	(15)		12	15	12	20	4
D19S433	12	14	15.2	16	12	16	12	15	5
TH01	6	9.3			6	9.3	6	9.3	2
FGA	19	24	26		19	21	20	21	5

Key:
Alleles that are shared between victim and S_1 or S_2 (green background).
Alleles that are found in the crime stain and not observed in any known individual (blue background, not applicable in this case).
Alleles that are below the detection threshold but appear to be distinct (bracketed).
Alleles that are found in the crime stain that match a known individual under *Hd* (victim) (red typeface).

Count the number of *unique alleles* in the 'set' in order to decide the number of contributors

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Step 3: Establish the minimum number of contributors for the 'preliminary' propositions

a) The swab is from a victim (*V*). There are two suspects (S_1,S_2) under *Hp*,

b) In this example, some loci have 5 unique alleles across sets hence there is a minimum of three individuals present under *Hp*.

c) A similar calculation can be made under *Hd* where the sets of genotypes formed by S_1,S_2 are not used, but in our rationale, it is convenient to anchor the minimum number of contributors on *Hp* and to assume equivalence (this is revisited later in the procedure).

d) Consequently, the preliminary propositions are formulated as *Hp*=*V*,S_1,S_2 and *Hd*=*V*,*U*,*U*

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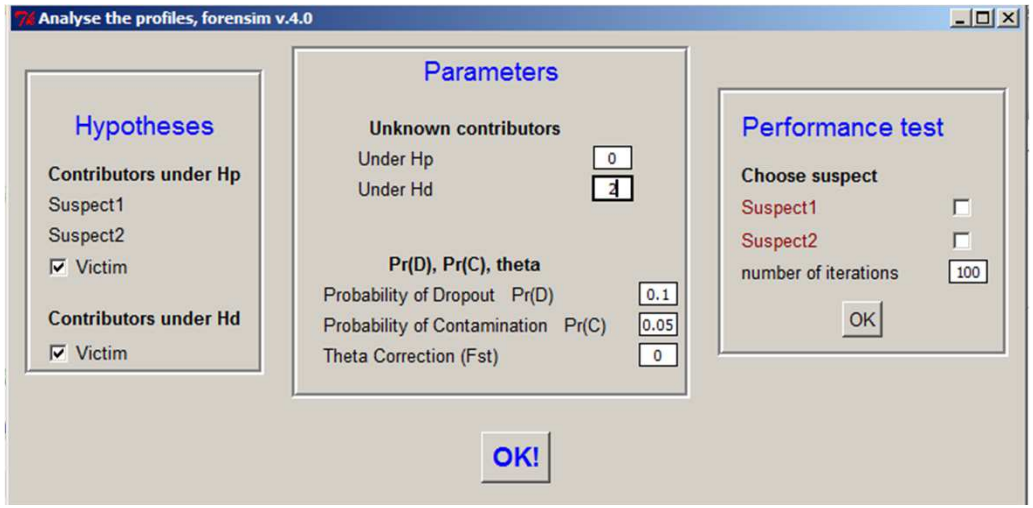
Step 4: Evaluate the first scenario

- The proposition under Hp is S1,S2,V
- The proposition under Hd is U1,U2,V
  - *Note we could also use U1, V under Hd – no need for Hd to agree on the same number of contributors*
  - (swab from female victim so this appears in Hp and Hd)



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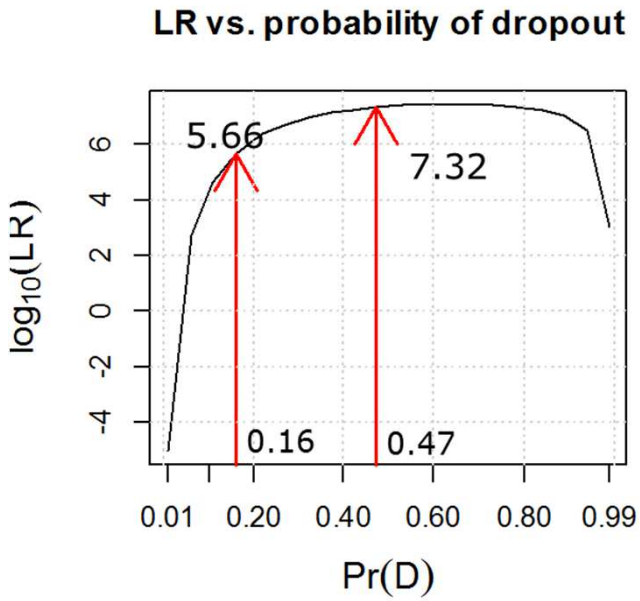
Sensitivity plot evaluation

- Plot the LR relative to all values of P(D)
- Calculate lower and upper bounds in order to decide a reasonable range
- Report the lowest value (to be conservative)



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We have got this far with our analysis

- Next we need to ask questions about whether the results themselves are robust?
- What sort of questions should you being asking?

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Step 5: Case re-evaluation and simplification of the propositions

Although a probative LR favouring *Hp* has resulted from the preliminary analysis, this has incorporated both suspects S_1 and S_2 under *Hp*.

However, the likelihood ratio itself does not provide any indication about the relative *weighting* of the two contributions provided by S_1, S_2 to the actual LR result.

Consequently, the next step in the analysis is to *dissect* the propositions into their constituents in order to establish the weighting and to establish the consequent probative value of the evidence per contributor under *Hp*.

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Step 5: Non-contributor testStep

- Why are we doing this?
- The process is *exploratory*
- So what will happen if we replace a suspect with a random man?
- We would expect the LR to be very low (an exclusion!!)
- Therefore, the non-contributor test is a measure of *robustness* and we consider this to be an important part of model *validation*

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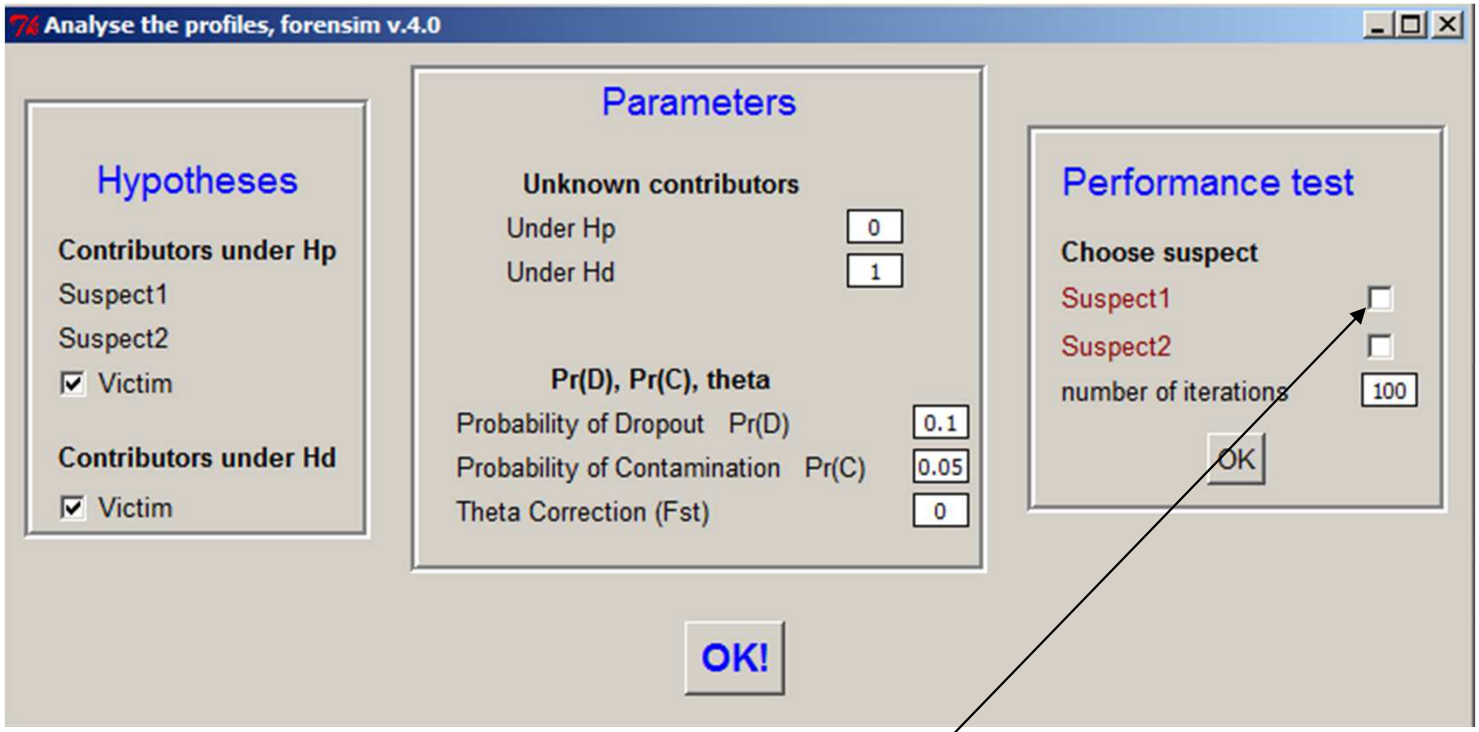
Run test



Click here – and click OK to start simulation

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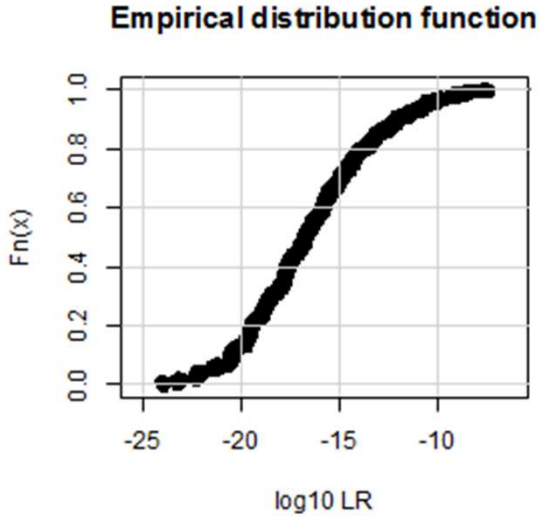
Comparison of non-contributor plots

There are two suspects – so we do two non-contributor plots:
a) replace S1 with r.m. (x1000) and
b) replace S2 with r.m. (x1000)

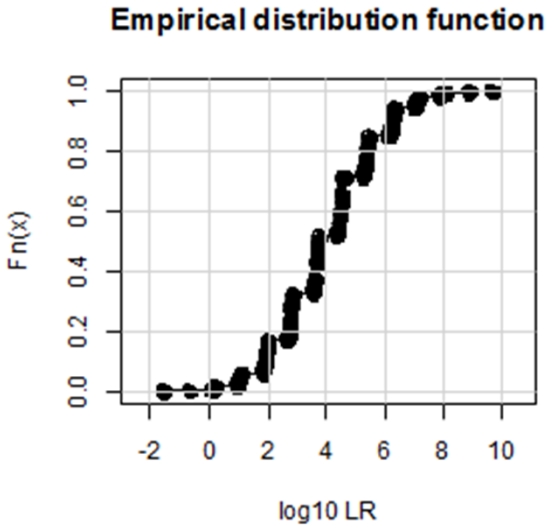
S1
a



quantile" "value"
"min" "-24.0269"
"0.01" "-23.2479"
"0.05" "-21.4325"
"0.5" "-16.7792"
"0.95" "-10.5699"
"0.99" "-8.4826"
"max" "-7.4584"

Original
LR=5.66

S2
b



"quantile" "value"
"min" "-1.591"
"0.01" "0.126"
"0.05" "1.0629"
"0.5" "3.7167"
"0.95" "7.0392"
"0.99" "7.9833"
"max" "9.6998"

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Step 5:Summarise the results

- The calculated LR(log10)= 5.6
- The non-contributor plot for S1 can be summarised using the one percentile, the median and the 99 percentile (-23,-16,-8)
- The non-contributor plot for S2 can be summarised in the same way: (+0.1,+3.7,+7.9)
- This means that the model is insensitive to S2 because the same result can be achieved with random man!!

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What does this mean?

- Beware complex propositions – the relative weightings of the S1,S2 ‘contributions’ are not reflected in the likelihood ratio
- Therefore complex propositions must be simplified and qualified before they can be reported
- The non-contributor plot is a useful adjunct to verify the likelihood ratio (define limitations of the model) and also provides an additional way to think about the results (court-friendly)

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Step 6: Simplify the propositions

- So far we don't have evidence for S2 under Hp
- So we need to think about different propositions in order to reevaluate the evidence
- There seems to be good evidence under Hp for S1

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New table with S1

Marker	Allele1	Allele2	Allele3	Allele4	S1	S1	No of unique alleles
AMEL	X	Y			X	Y	2
D3S1358	14	16	17	(15)	16	17	3
VWA	16	17	18	19	16	18	4
D16S539	11	12	13	15	12	13	4
D2S1338	17	19	20	(24)	19	20	4
D8S1179	9	10	13	14	9	13	4
D21S11	29	31	32		28	32	4
D18S51	12	16	(15)		12	15	3
D19S433	12	14	15.2	16	12	16	4
TH01	6	9.3			6	9.3	2
FGA	19	24	26		19	21	4

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Analysis

Visual examination of the evidence (table 2) revealed that S_1 has more matching alleles than S_2; furthermore the crime stain could be explained under *Hp* if it was a simple mixture of *V* and S_1 (with three *dropped-out* alleles).

Individual S_2 is not required at all in the analysis, since there are no missing alleles observed in the crime stain ($Hp=V,S_1$).

Although the number of unique alleles reduces the number of contributors to two, in order to be consistent, three contributors are evaluated and the propositions are simplified to: $Hp=S_1,V,U$ and $Hd=V,U,U$.

(*note the LR is much larger if two contributors are analysed under Hp and Hd – data not shown, hence the choice of three contributors is demonstrably conservative*).

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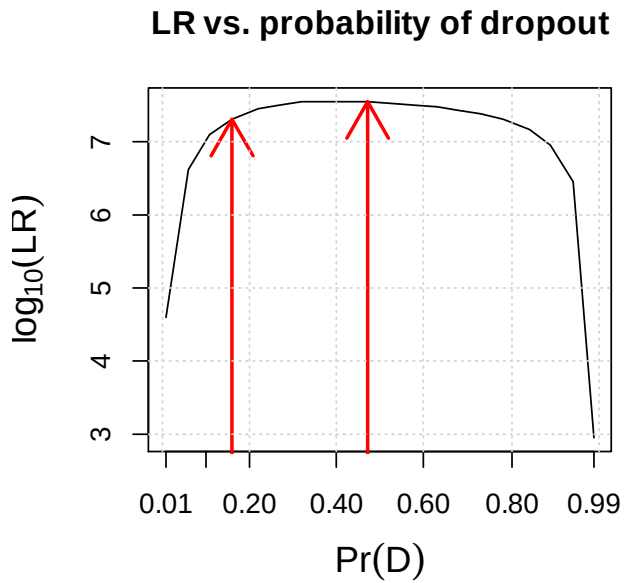
New proposition: Hp=S1,V,U and Hd=V,U,U

The new $\log_{10}(LR_{min})$= 7.32; $Pr(D_{min})$=0.16



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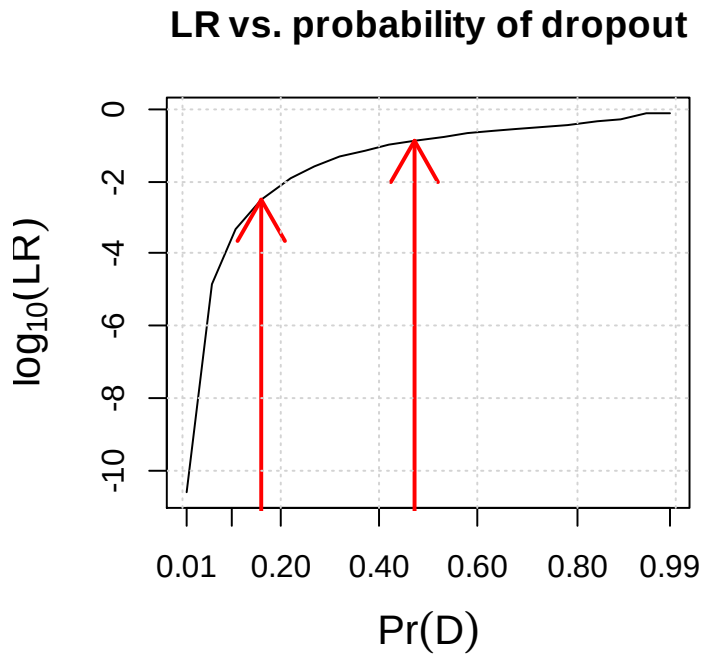
Now determine the S2 effect

Hp=S_2,*V*,*U*; *Hd*=*V*,*U*,*U*.

$Pr(D_{min})=0.16$

$\log_{10}(LR_{min})$= -2.6 which is clearly 'exclusionary'



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Step 7: Non-contributor performance (Np) tests summary

Np tests can be used to support the conclusion that evidence supporting S_1 is 'inclusionary' whereas evidence supporting S_2 is 'exclusionary'

	Three person mixture			Non-contributor performance
Hp	*Hd*	Random man substituted	$\log_{10}$(LR)	percentiles
S_1,S_2,*V*	*V*,*U*,*U*	S_1	5.5	(-21,-15,-7)
S_1,S_2,*V*	*V*,*U*,*U*	S_2	5.5	(+0.17,+4.2,+8.2)
S_1,*V*,*U*	*V*,*U*,*U*	S_1	7.2	(-10,-5,+0.14)
S_2,*V*,*U*	*V*,*U*,*U*	S_2	-3	(-10,-5,+0.14)

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Principles to follow when evaluating complex sets of hypotheses Conditioning rules (a)

- Conditioning hypotheses are defined by the casework circumstances
- Remember to evaluate the hypotheses based on the number of contributors derived from the unique number of alleles in the 'set' observed in the epg: i.e. the sum of alleles of known contributors and the sum of alleles of the crime-stain(s) under Hp (to maximise)
- Do not use the *drop-in* principle to 'explain away' additional contributors

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Principles to follow when evaluating complex sets of hypotheses Conditioning rules (b)

- If there are two or more ‘suspects’ under Hp then the hypothesis should be simplified i.e. evaluate: S1,V,U in addition to S1,S2,V
- It is important to explore the likelihood ratio by use of the non-contributor plot.
- In the S1,S2,V example we show that the LR is very insensitive to S2 (random man still gives a high LR)

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Summary of results

- Case circumstances
  - Both S1 and S2 are suspects of sexual assault and a sample is taken from the victim. We condition on the victim under Hd
  - No evidence for S2 in the crime stain [even though a three person evaluation with S1,S2 under Hp gives a high LR= log10(5)
  - Advice: Simplify propositions if there are two suspects - always evaluate them separately.

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