Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

The Clayton Rules

Oskar Hansson

Statistical methods in forensic genetics 7-10 October 2013, Copenhagen

(slides adapted from John Butler)
(Basic Principles in Forensic DNA Evidence Interpretation, ISFG 2013, Melbourne)

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
2. Scientists should be trained in and use LRs
3. Methods to calculate LRs of mixtures are cited
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
7. Allele dropout to explain evidence can only be used with low signal data
8. No statistical interpretation should be performed on alleles below threshold
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Steps in the Interpretation of Mixtures

(Clayton *et al.* 1998)

Step #1 Identify the Presence of a Mixture

↓

Step #2 Designate Allele Peaks

↓

Step #3 Identify the Number of Potential Contributors

↓

Step #4 Estimate the Relative Ratio of the Individuals Contributing to the Mixture

↓

Step #5 Consider All Possible Genotype Combinations

↓

Step #6 Compare Reference Samples

ELSEVIER

Forensic Science International

Analysis and interpretation of mixed forensic stains using DNA STR profiling

T.M. Clayton[a,*], J.P. Whitaker[a], R. Sparkes[b], P. Gill[b]

[a]Forensic Science Service, Wetherby Laboratory, Sandbeck Way, Audby Lane, Wetherby, West Yorkshire LS22 4DN, UK

[b]Forensic Science Service, Priory House, Gooch Street North, Birmingham B36QQ, UK

Received 15 May 1997; received in revised form 9 October 1997; accepted 27 October 1997

Clayton *et al.* (1998) *Forensic Sci. Int.* 91:55-70

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Step #1: Is a Mixture Present in an Evidentiary Sample?

- Examine the number of peaks present in a locus
  - More than 2 peaks at a locus (except for tri-allelic patterns at perhaps one of the loci examined)
- Examine relative peak heights
  - Heterozygote peak imbalance <60%
  - Peak at stutter position >15%
- Consider all loci tested

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

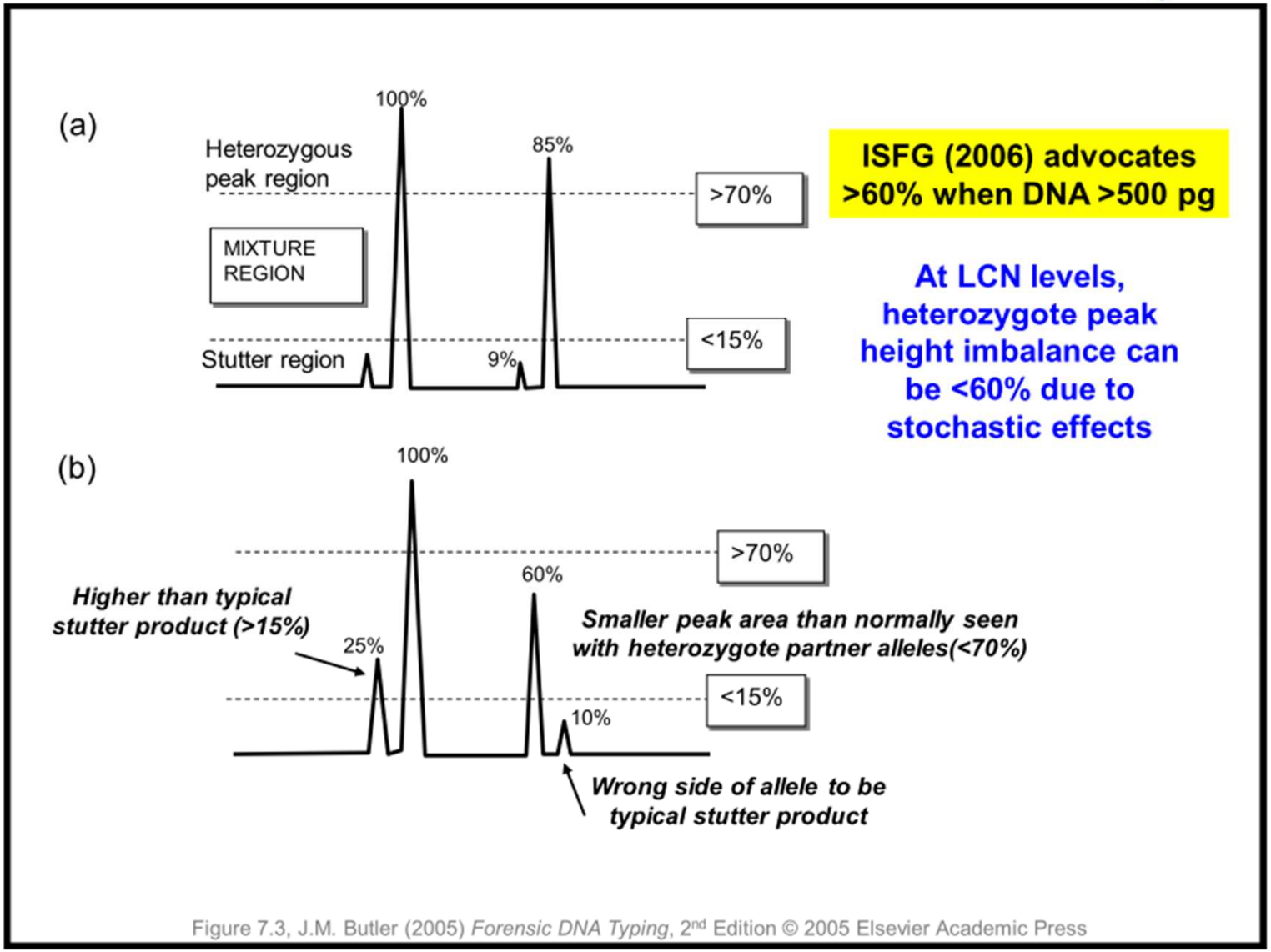


Figure 7.3, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Step #2: Designate Allele Peaks

- Use regular data interpretation rules to decipher between true alleles and artifacts
- Use stutter filters to eliminate stutter products from consideration (although stutter may hide some of minor component alleles at some loci)
- Consider heterozygote peak heights that are highly imbalanced (<60%) as possibly coming from two different contributors

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Step #3: Identifying the Potential Number of Contributors

- **Important for some statistical calculations**
- Typically if 2, 3, or 4 alleles then 2 contributors
- If 5 or 6 alleles per locus then 3 contributors
- If >6 alleles in a single locus, then >4 contributors

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Step #4: Estimation of Relative Ratios for Major and Minor Components to a Mixture

- Mixture studies with known samples have shown that the mixture ratio between loci is fairly well preserved during PCR amplification
- Thus it is generally thought that the peak heights (areas) of alleles present in an electropherogram can be related back to the initial component concentrations
- Start with loci possessing 4 alleles…

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Step #5: Consider All Possible Genotype Combinations

Table 3
Pairwise combinations of two, three and four alleles

Four alleles (a,b,c,d)		Three alleles (a,b,c)		Two alleles (a,b)	
a,b	c,d	a,a	b,c	a,a	a,b
a,c	b,d	b,b	a,c	a,b	a,b
a,d	b,c	c,c	a,b	a,a	b,b
c,d	**a,b**	a,b	a,c	a,b	b,b
b,d	**a,c**	b,c	a,c	**a,b**	**a,a**
b,c	**a,d**	a,b	b,c	**b,b**	**a,a**
		b,c	**a,a**	**b,b**	**a,b**
		a,c	**b,b**		
		a,b	**c,c**		
		a,c	**a,b**		
		a,c	**b,c**		
		b,c	**a,b**		

Key: bold entries represent reciprocal combinations.

Clayton *et al. Forensic Sci. Int.* 1998; 91:55-70

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Step #6: Compare Reference Samples

- If there is a suspect, a laboratory must ultimately decide to include or exclude him…
- **If no suspect is available for comparison, does your laboratory still work the case?** (Isn't this a primary purpose of the national DNA database?)
- Victim samples can be helpful to eliminate their allele contributions to intimate evidentiary samples and thus help deduce the perpetrator

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Presentations from the ISFG workshops

Basic Principles in Forensic DNA Evidence Interpretation

Advanced Topics in Forensic DNA Evidence Interpretation

http://www.cstl.nist.gov/strbase/pub_pres/

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Low Template DNA

Oskar Hansson

Statistical methods in forensic genetics 7-10 October 2013, Copenhagen

(slides adapted from Peter Gill)

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

What is Low Copy Number?

- Let's make a list of what LCN is not
  - Its not related to an overall quantity of DNA (such as 200pg)
  - Its not restricted to 'touch DNA'
  - Its not related to any particular technique
- NY court found it to be a simple extension of an existing technique
- R. v. Reed accepted that the 34 cycle definition was not relevant to any definition of LT-DNA
- Why can't a definition be adduced?

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Stochastic variation

- In a heterozygous sample, one allele is amplified more than the other
- Leads to heterozygous imbalance or allele drop-out
  - Good quality DNA will always give heterozygous balance >60% i.e. both target alleles are amplified with similar efficiency
- Much more pronounced with low level DNA as there is less template DNA
- If one target gets amplified more in the first rounds of PCR then it becomes preferentially amplified in later rounds

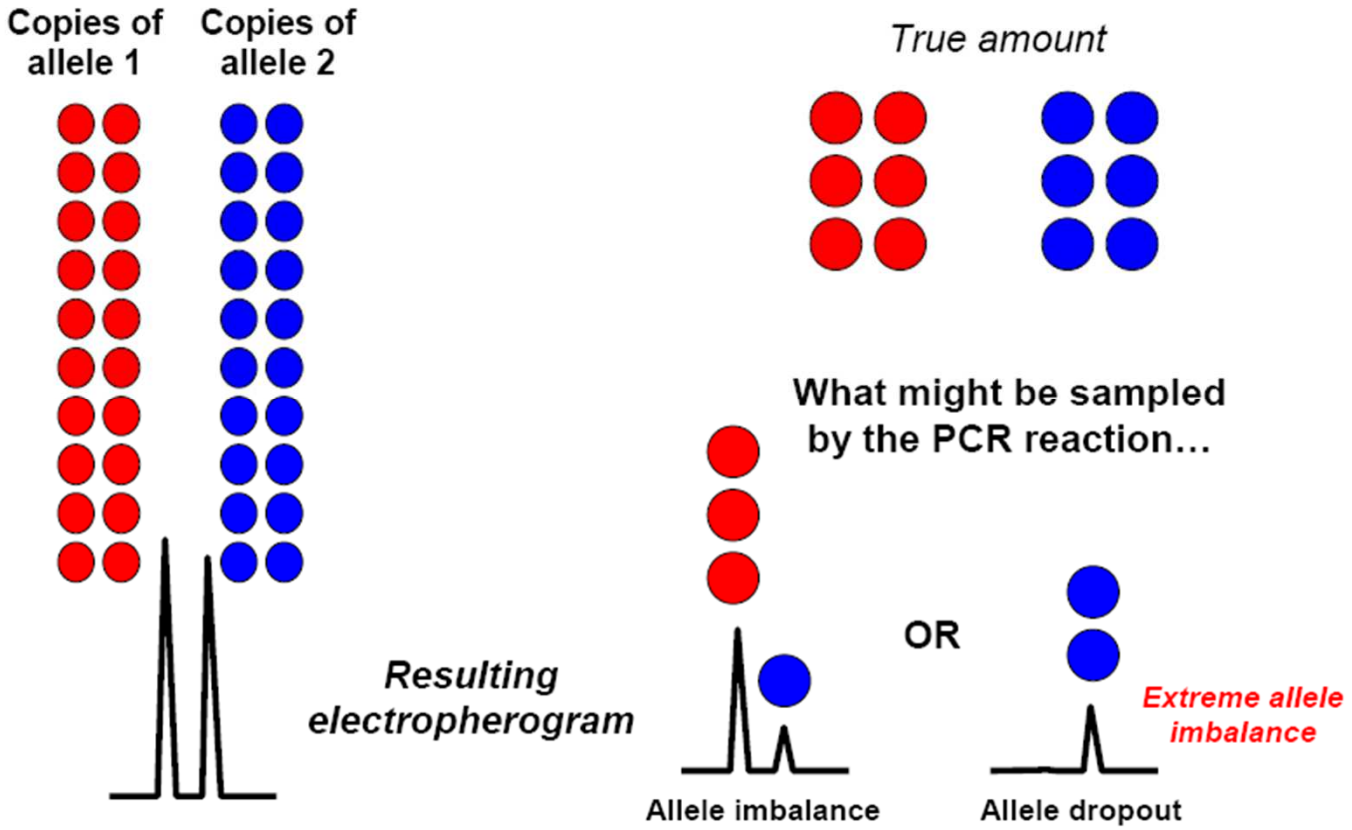
SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Heterozygous imbalance OR allele drop-out
(from J Butler, NIST)

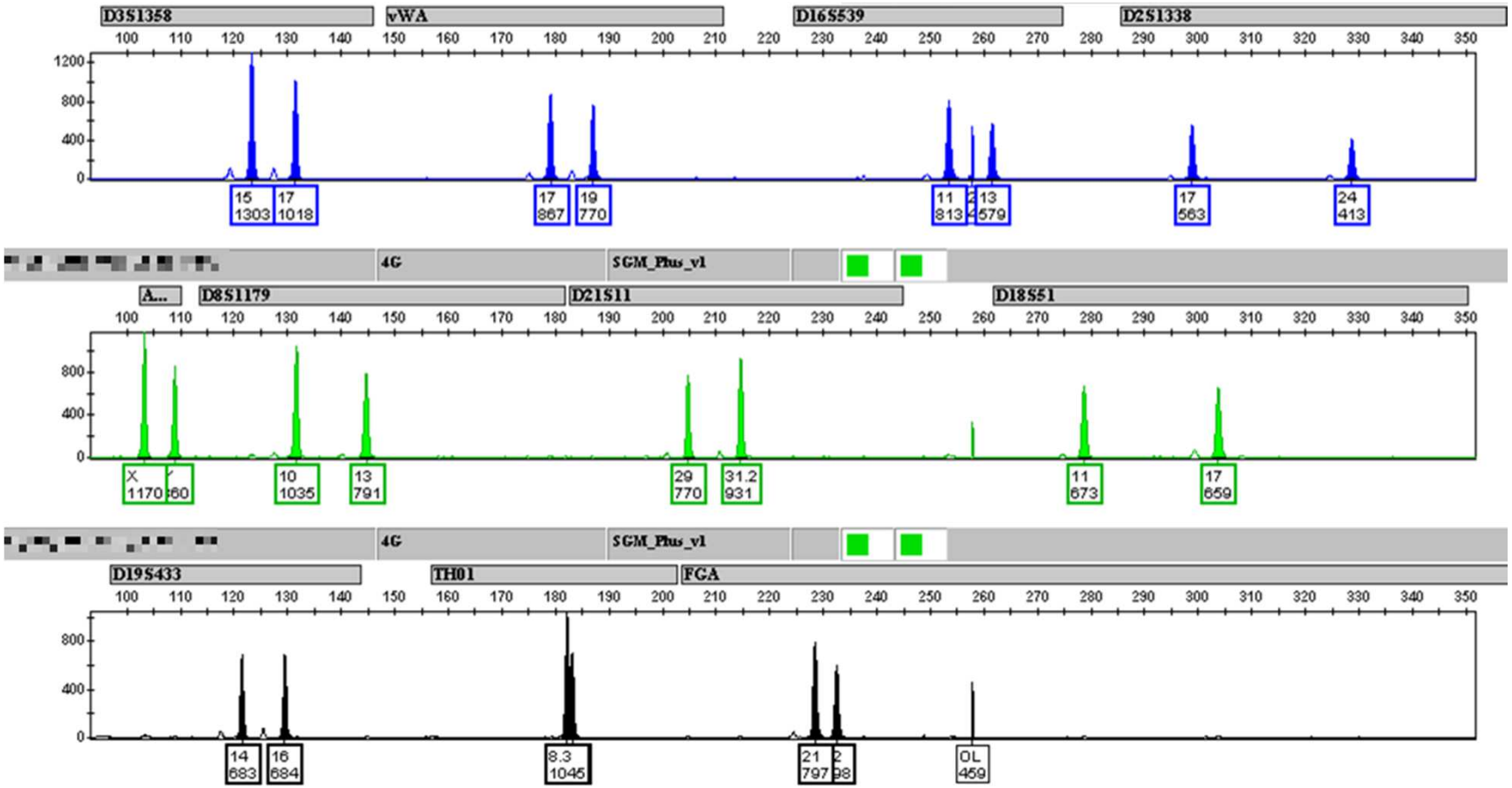


EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Example of good level DNA

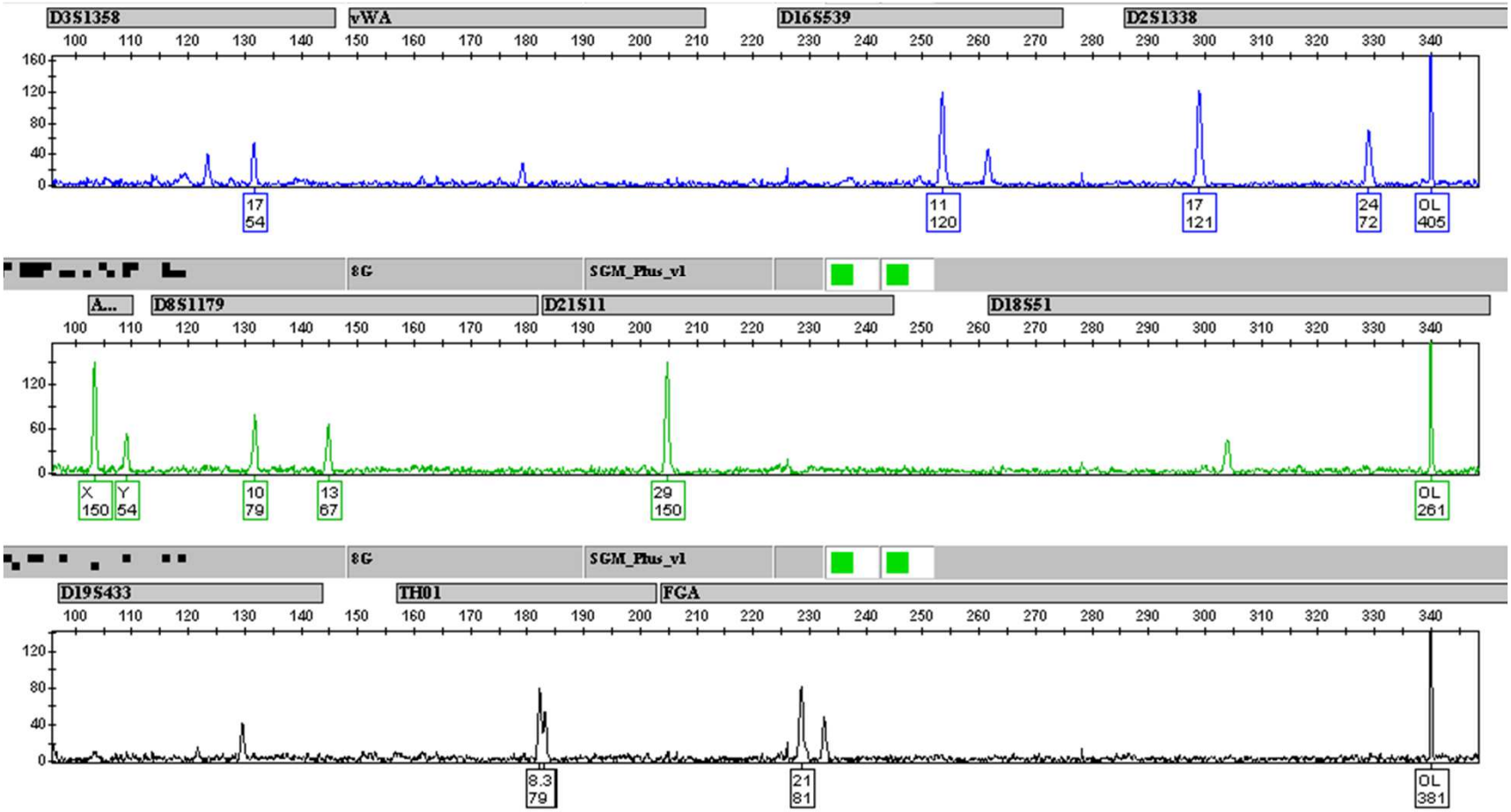


EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Example of low level DNA



EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

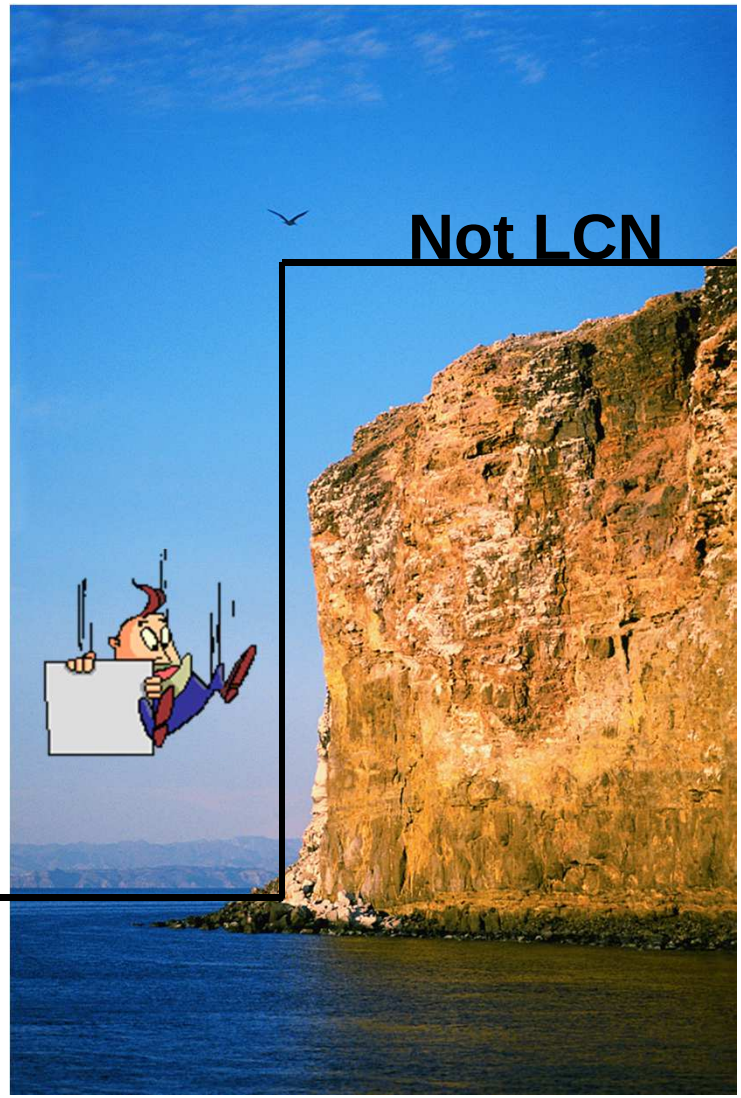
EUROFORGEN Network of Excellence

Illogical use of thresholds

- Falling off the cliff

For example if we have a rule that states:
150 RFU – This is conventional
versus 149 RFU – This is LCN

- There is nothing in between

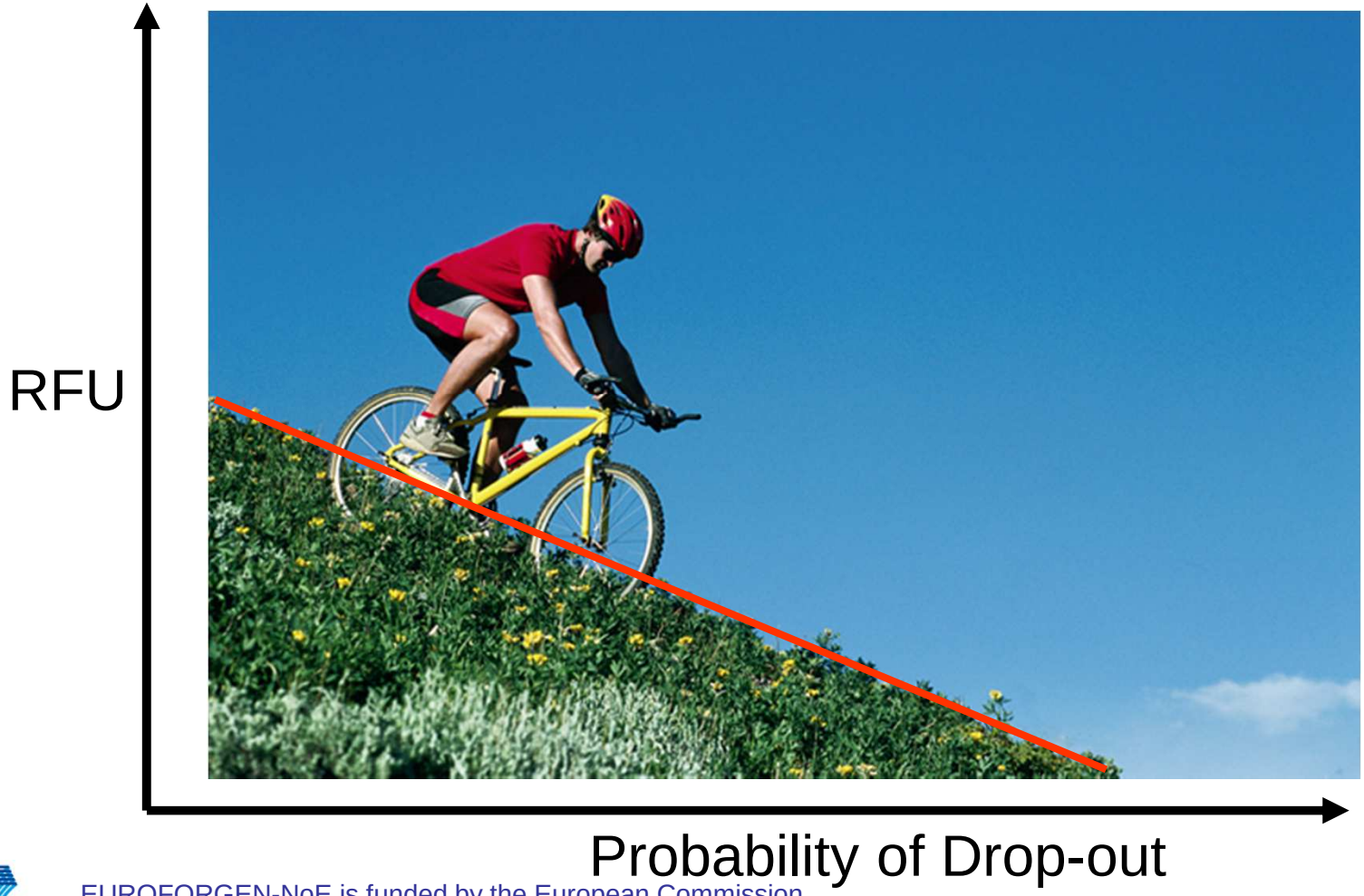


EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

In reality it's a gentle ride downhill



EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Continuum of change

- The peak height rules break down when the quantity of DNA becomes very low – in particular the Hb guideline will no longer hold true
- Allele drop-out can lead to a heterozygous locus being genotyped as a homozygous locus
  - In standard DNA profiling, a homozygous peak height of 150 RFU is often used (stochastic threshold) i.e. single peaks <150 RFU are labeled ‘F’ indicating allele drop-out may have occurred

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

This is why we prefer a universal method

Forensic Science International: Genetics 4 (2010) 221–227

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

A universal strategy to interpret DNA profiles that does not require a definition of *low-copy-number*

Peter Gill [a,b,*], John Buckleton [c]

[a] University of Strathclyde, Glasgow, UK
[b] Institute of Legal Medicine, University of Oslo, Oslo, Norway
[c] ESR, Auckland, New Zealand

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Papers outlining heterozygous balance

Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. (2002) TWGDAM validation of AmpFlSTR PCR amplification kits for forensic DNA casework. J. Forensic Sci. 47(1): 66-96.

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. (2004) Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. J. Forensic Sci. 49(6): 1265-1277.

L.A. Dixon, C.M. Murray, E.J. Archer, A.E. Dobbins, P. Koumi & P. Gill (2005) Validation of a 21-locus autosomal SNP multiplex for forensic identification purposes. For. Sci. Int. 154 (1): 62-77

Gill, P., Sparkes, R. and Kimpton, C. (1997). "Development of guidelines to designate alleles using an STR multiplex system." *Forensic Sci Int* **89**(3): 185-197

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Degradation

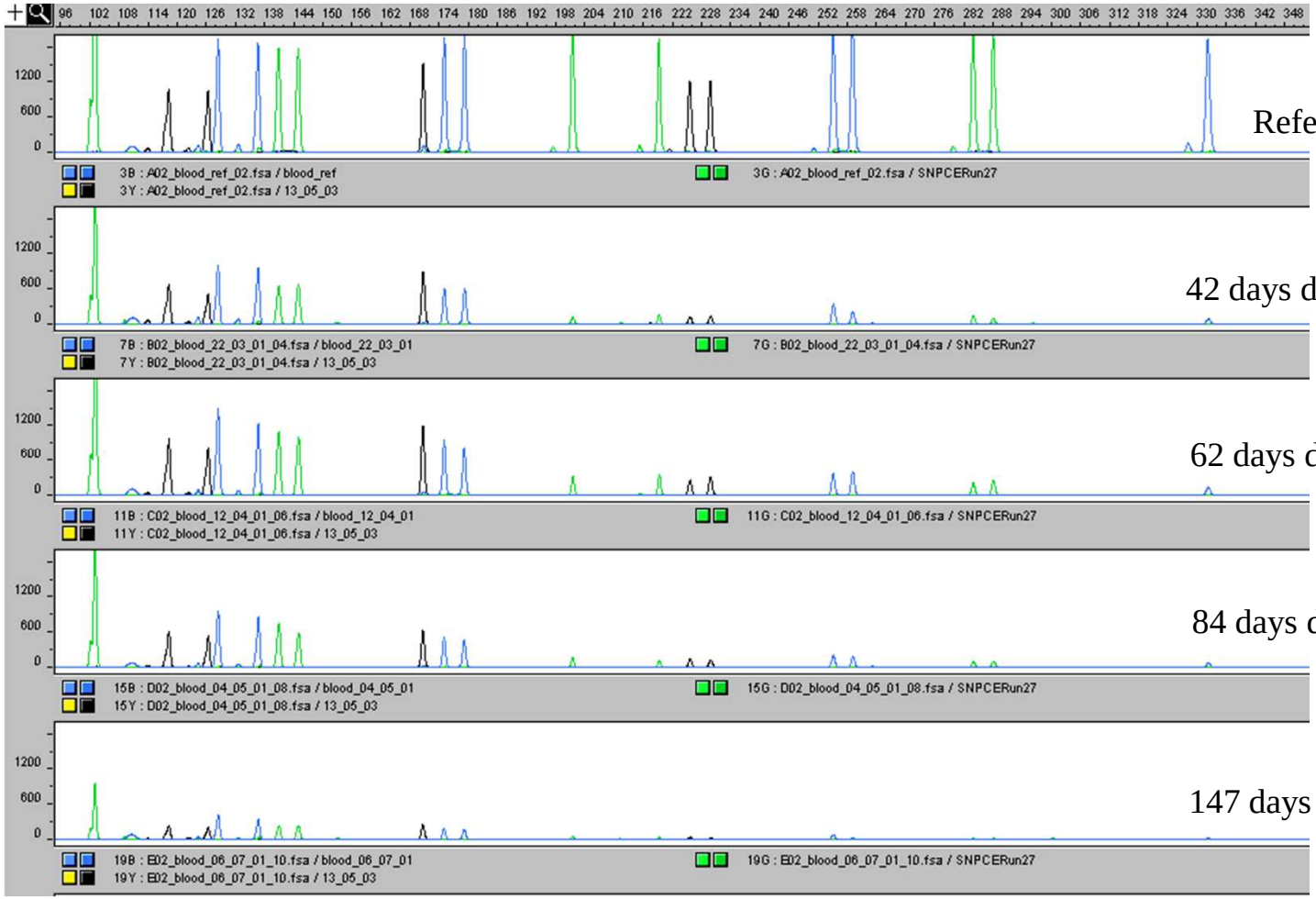
- Occurs with fragmented / degraded DNA as there are more of the small target molecules available for amplification
- Leads to a distinctive slope in peak heights across the profile

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Effect of degradation



EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Allele drop-in

- A contamination event resulting in only one or two foreign alleles
- Independent from gross contamination in that it comes from different sources

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Contamination

- Gross contamination is identified as being from a single contributing source
- Dependent on transfer event as to when contamination occurred
- Could be pre-incident or post-incident

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

New methods

- Incorporate probability of dropout and dropin
- Uses statistical theory that is well established
- The theory can be used to evaluate complex mixtures
- No limitation on number of contributors
- No limitation on number of replicates that can be combined to form a single LR

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Introduction to the LRmix program

of the Forensim R package

Oskar Hansson

Statistical methods in forensic genetics 7-10 October 2013, Copenhagen

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

For news updates subscribe to
forensimnews@gmail.com

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the R software

www.cran.r-project.org

The Comprehensive R Archive Network

CRAN
Mirrors
What's new?
Task Views
Search

About R
R Homepage
The R Journal

Software
R Sources
R Binaries
Packages
Other

Documentation
Manuals
FAQs
Contributed

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

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the R software

R for Windows

CRAN
Mirrors
What's new?
Task Views
Search

About R
R Homepage
The R Journal

Software
R Sources
R Binaries
Packages
Other

Documentation
Manuals
FAQs
Contributed

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.

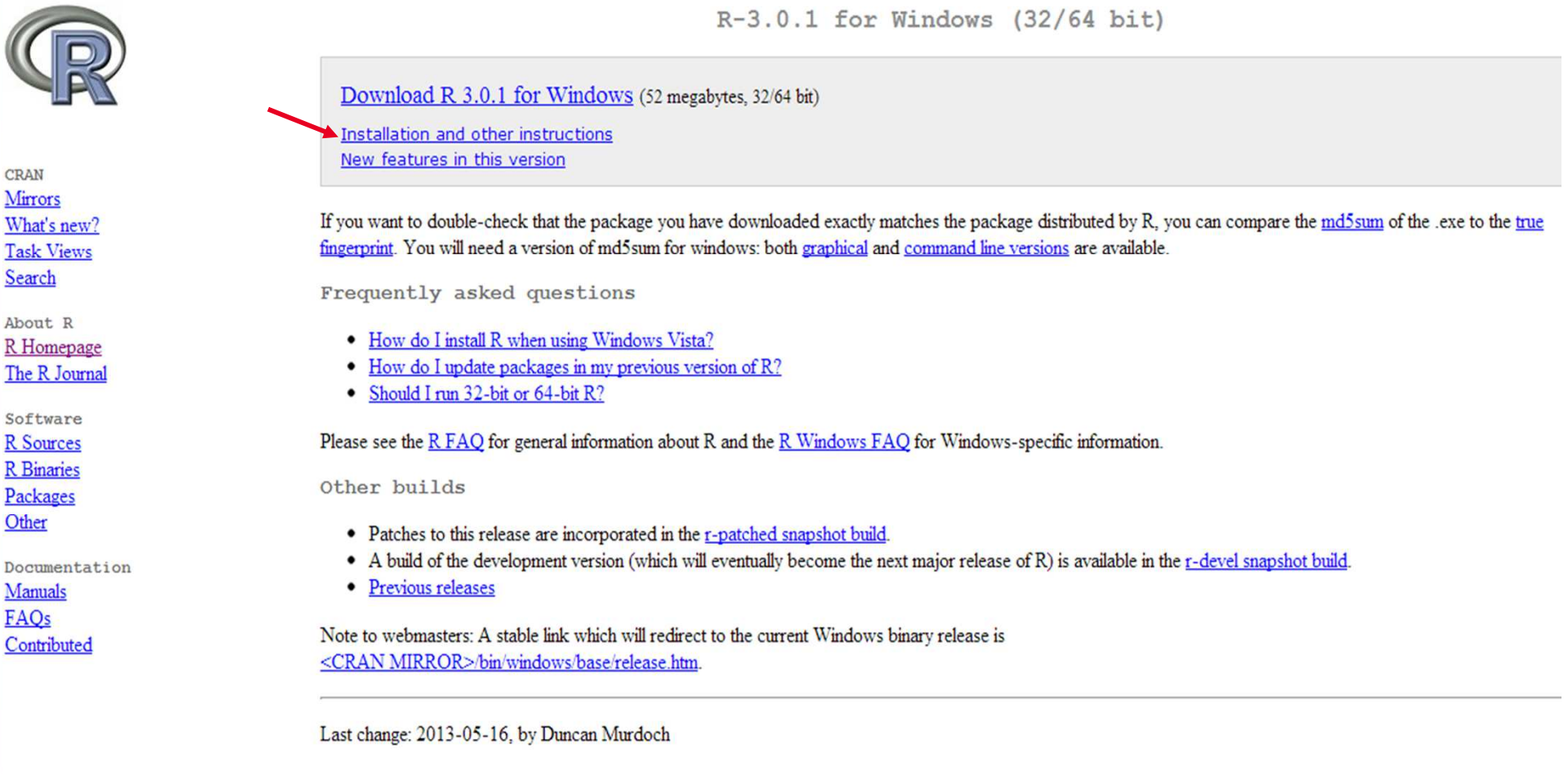
SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the R software



CRAN
Mirrors
What's new?
Task Views
Search

About R
R Homepage
The R Journal

Software
R Sources
R Binaries
Packages
Other

Documentation
Manuals
FAQs
Contributed

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

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the R software

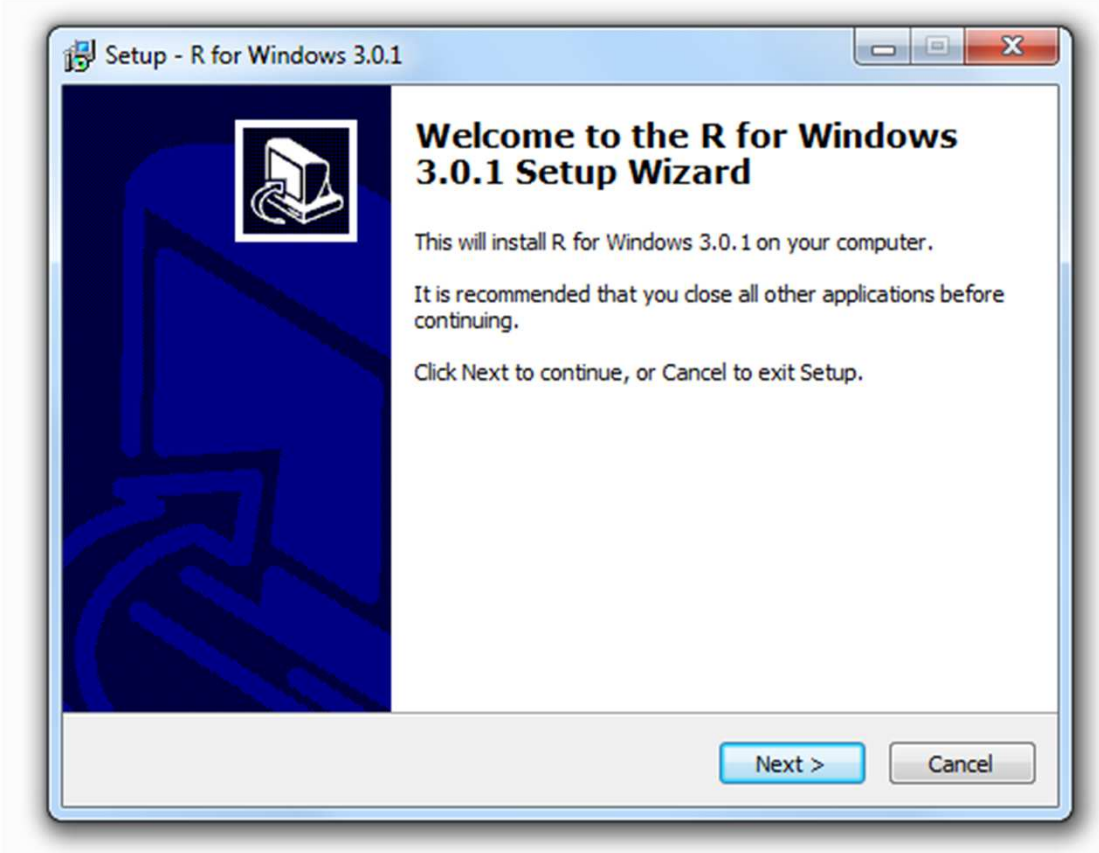
- An executable file will be downloaded.
- R.3.0.1.exe (or newer version)
- Simply click and follow the instructions!

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the R software



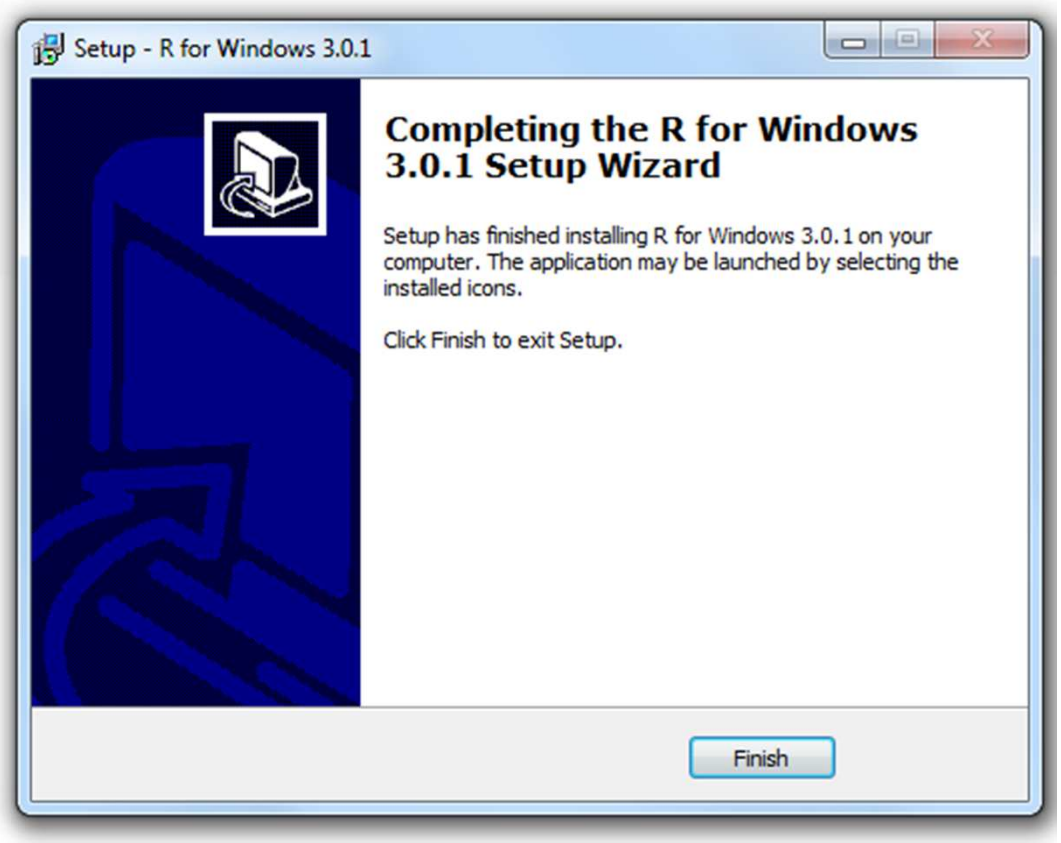
Press ‘next’ until...

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the R software



EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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.

SEVENTH FRAMEWORK PROGRAMME

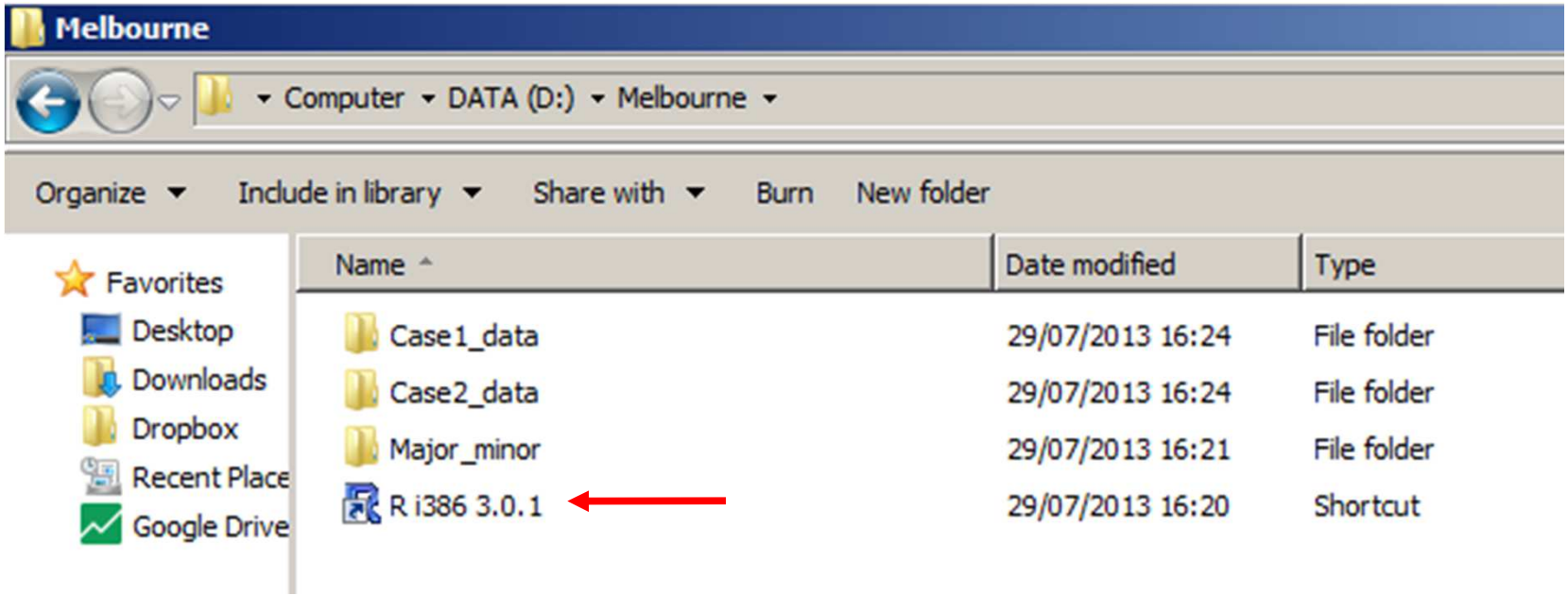
EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

You are ready to launch R

Double-Click blue icon.

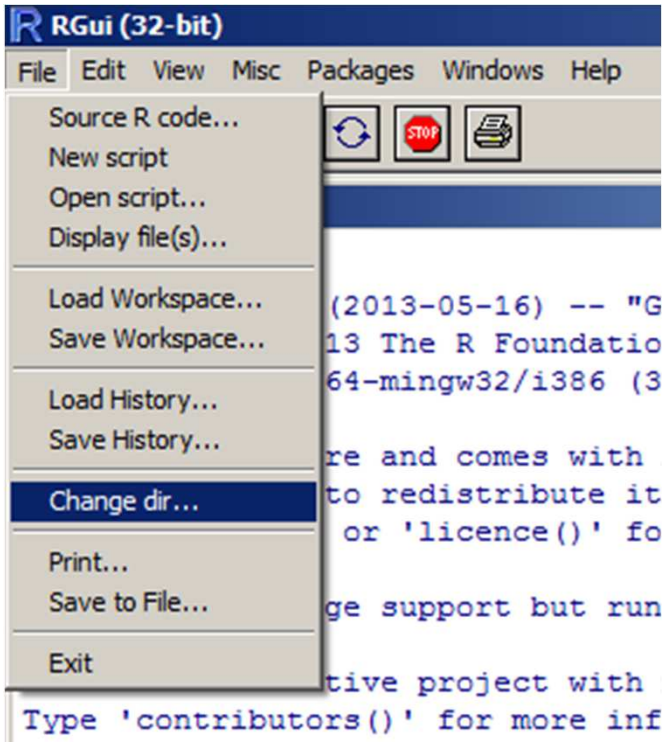


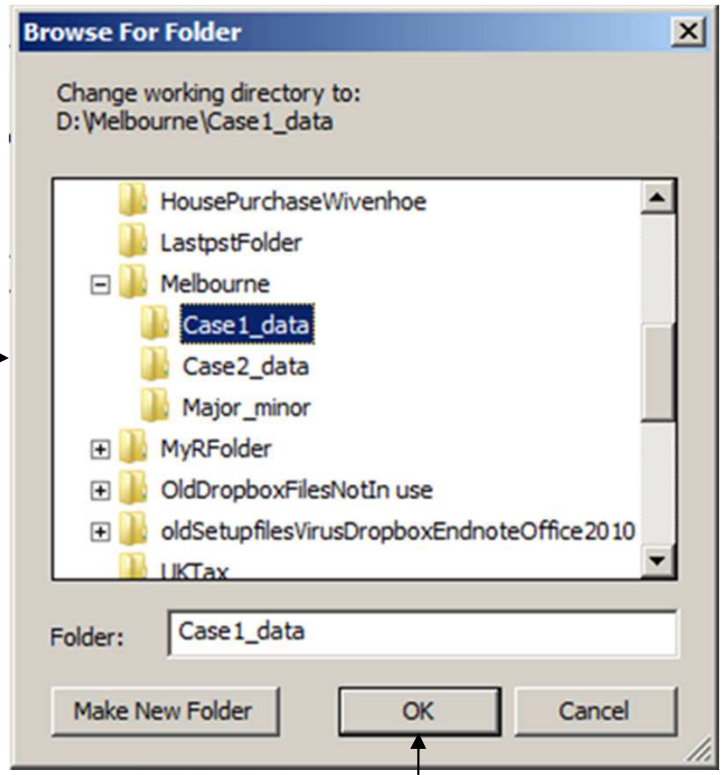
EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Set directory to your folder





Press OK to set directory

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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

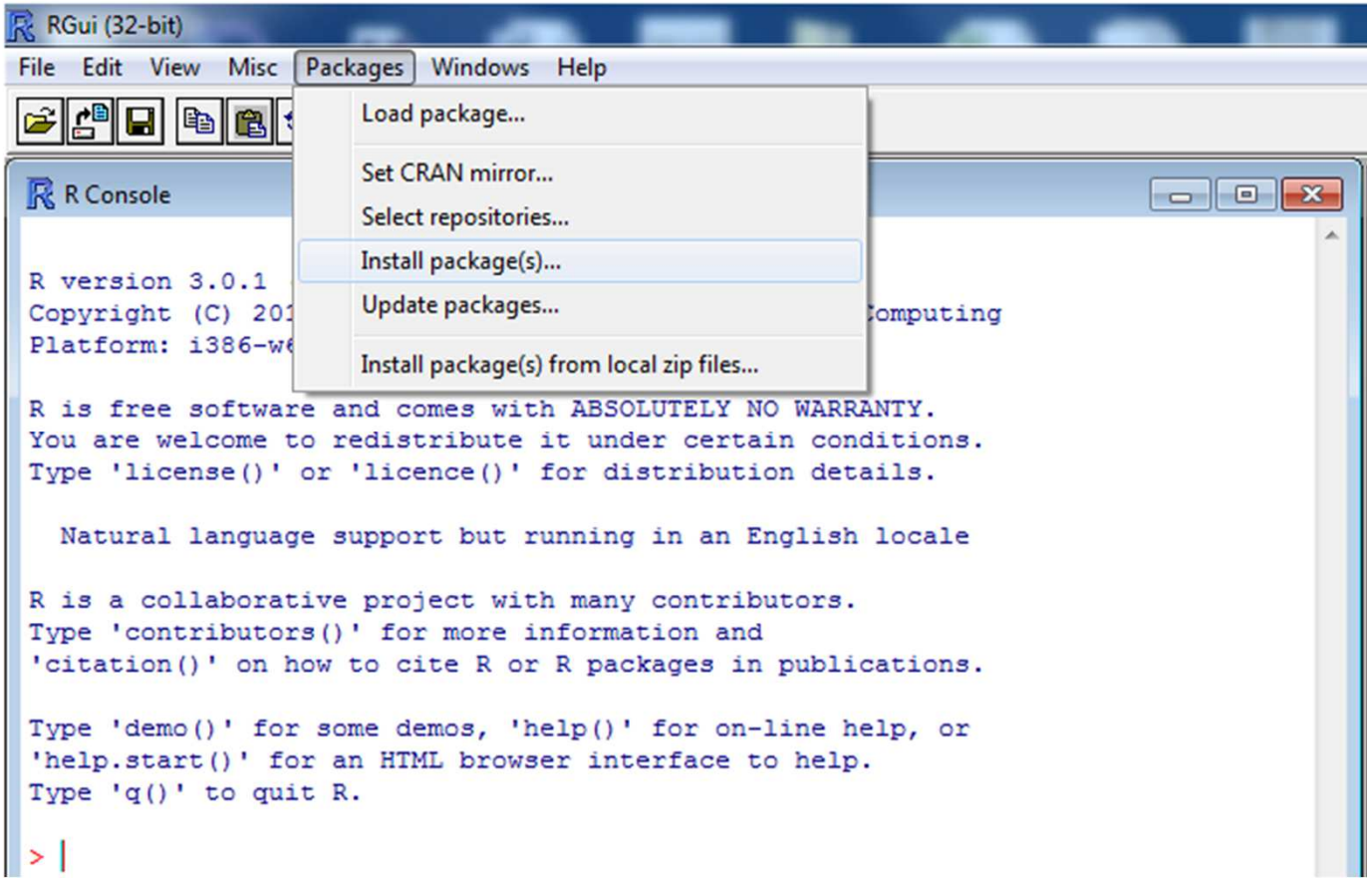
- Option 2: Install the package manually (no Internet connection)
  - ☛ Refer to LRmix tutorial online

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the Forensim package



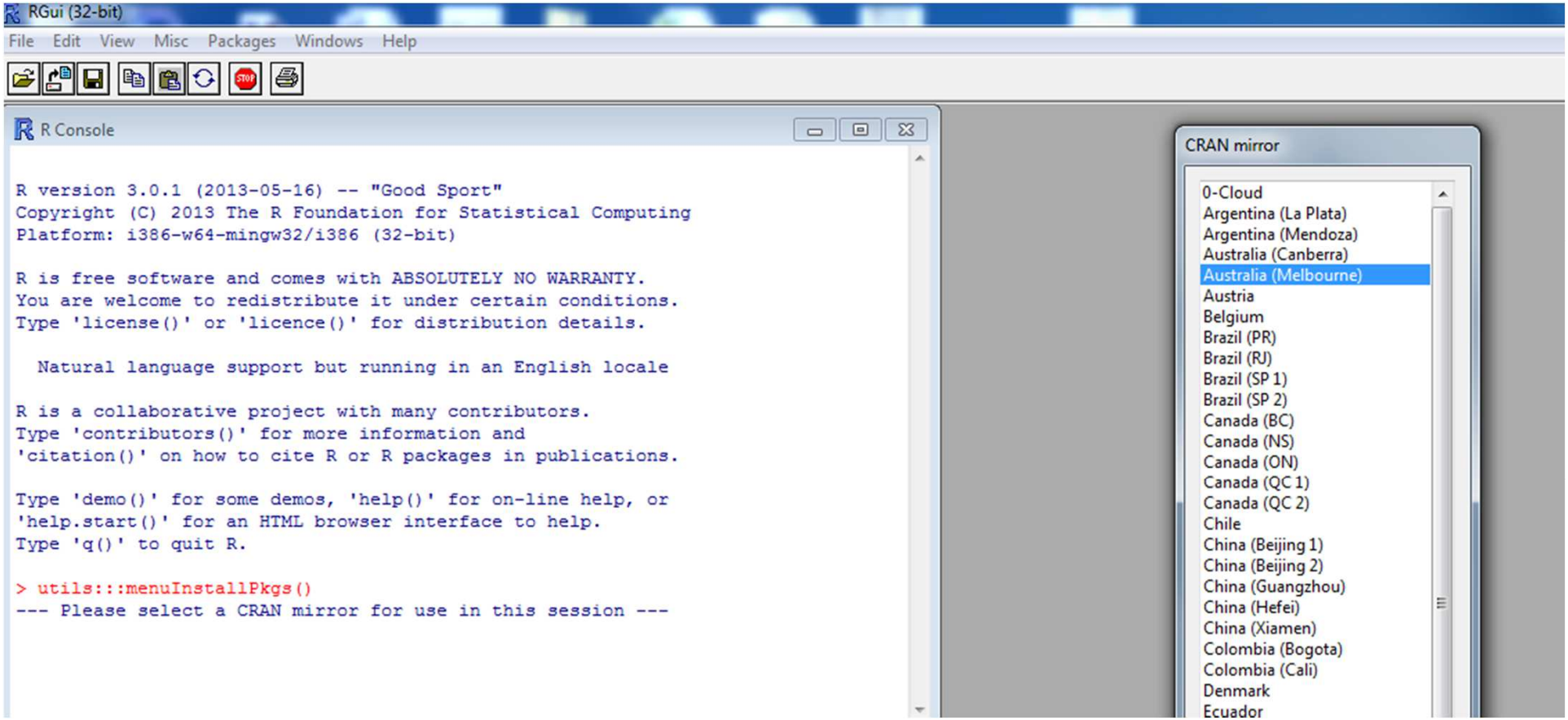
EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Install the Forensim package

Choose a mirror that is geographically close to you

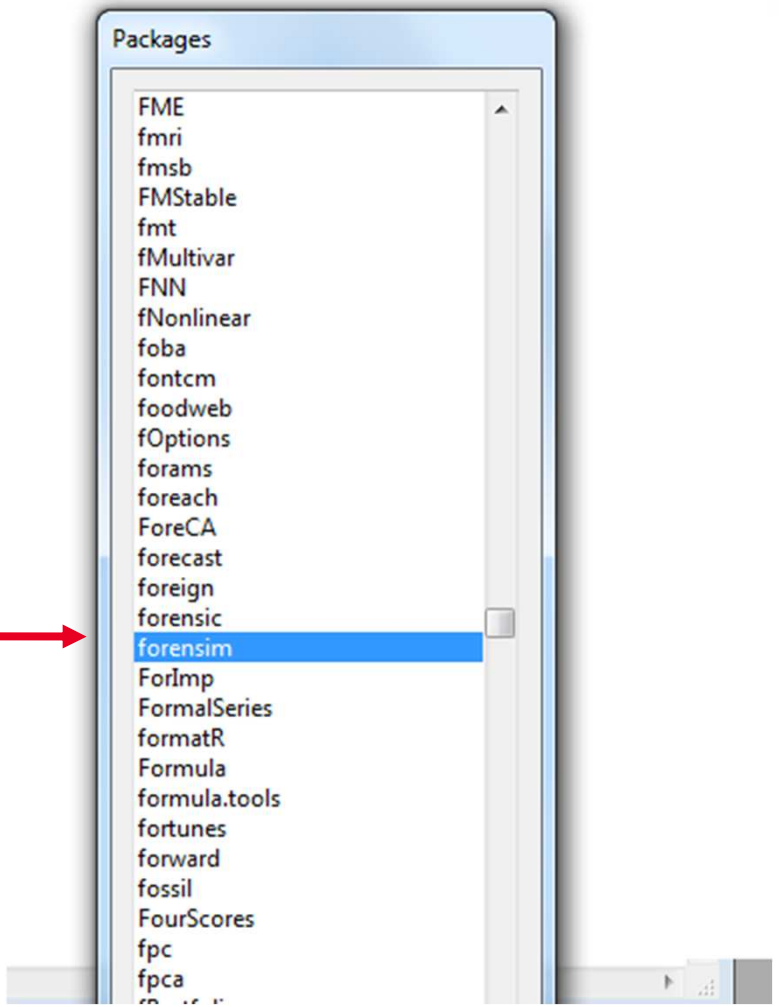


EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN

Choose package forensim

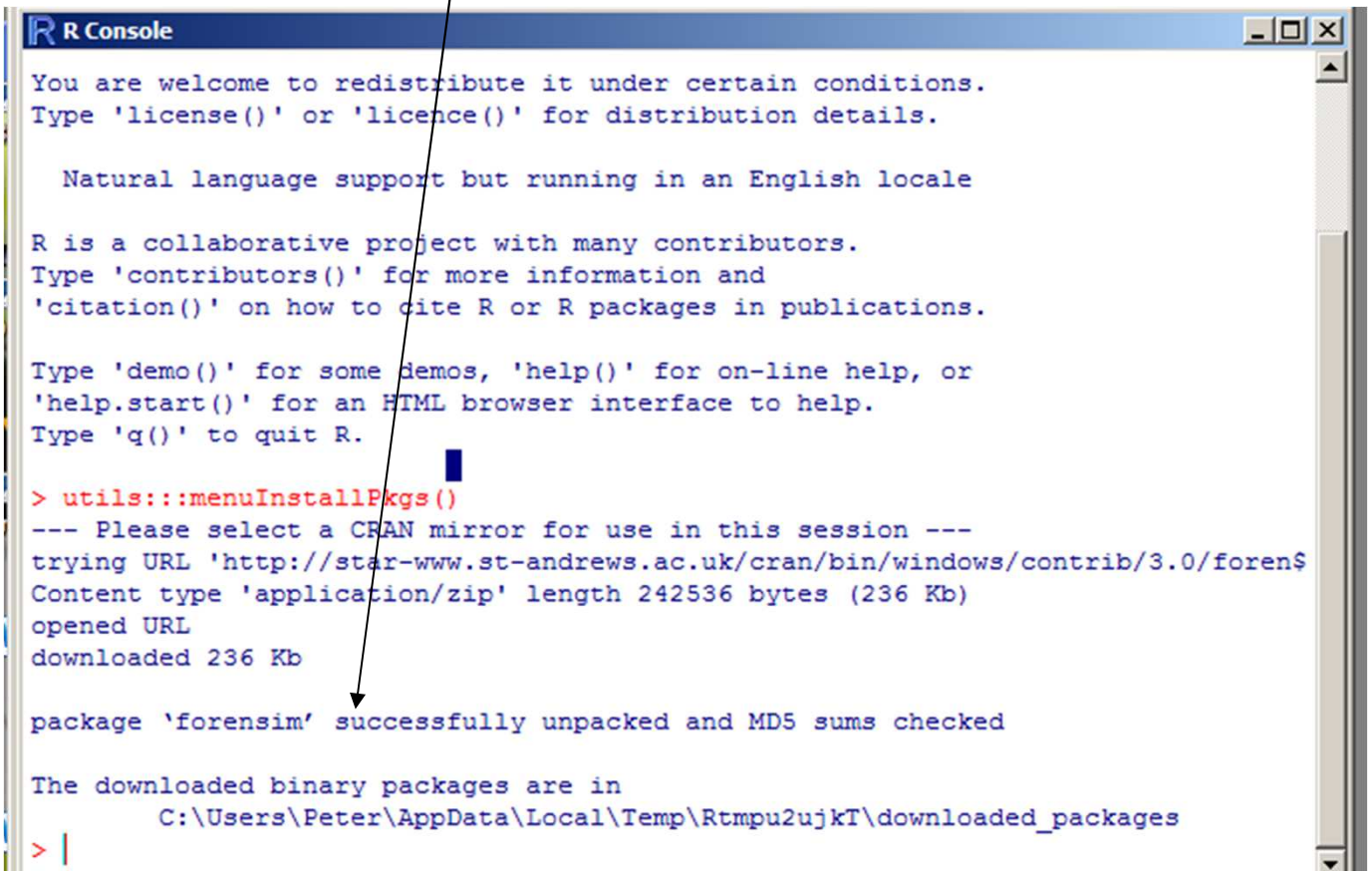


EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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



EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

- 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

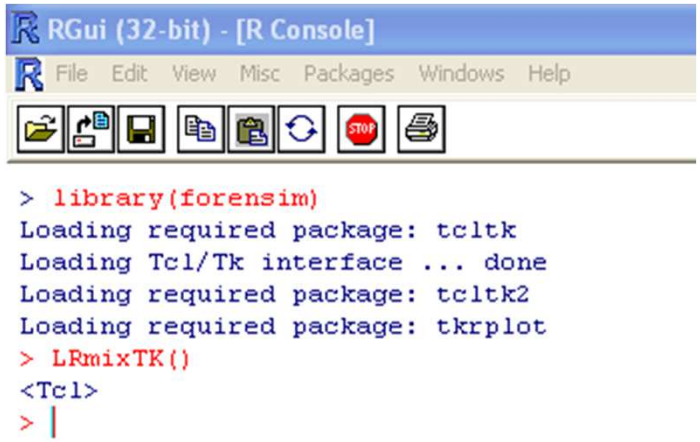
EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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.

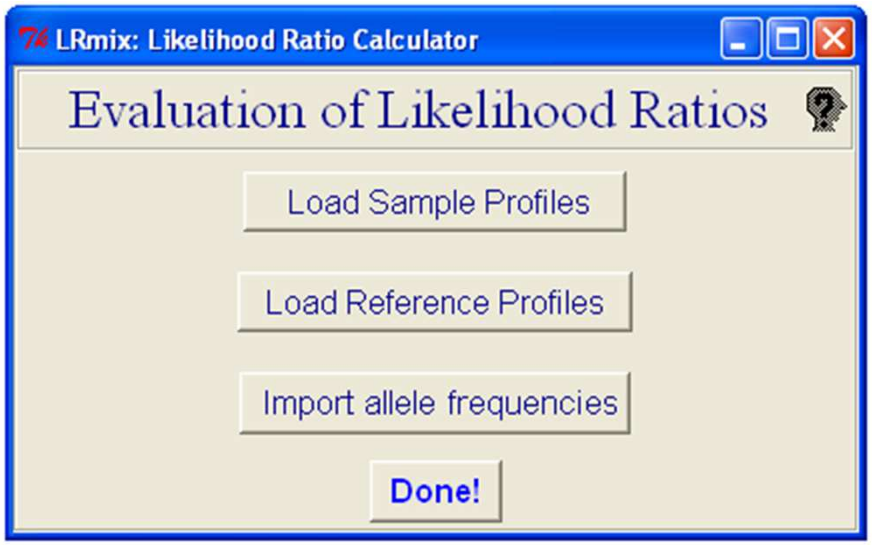
NB! Commands in R are case sensitive.

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

The main LRmix interface



EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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)

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

A case example

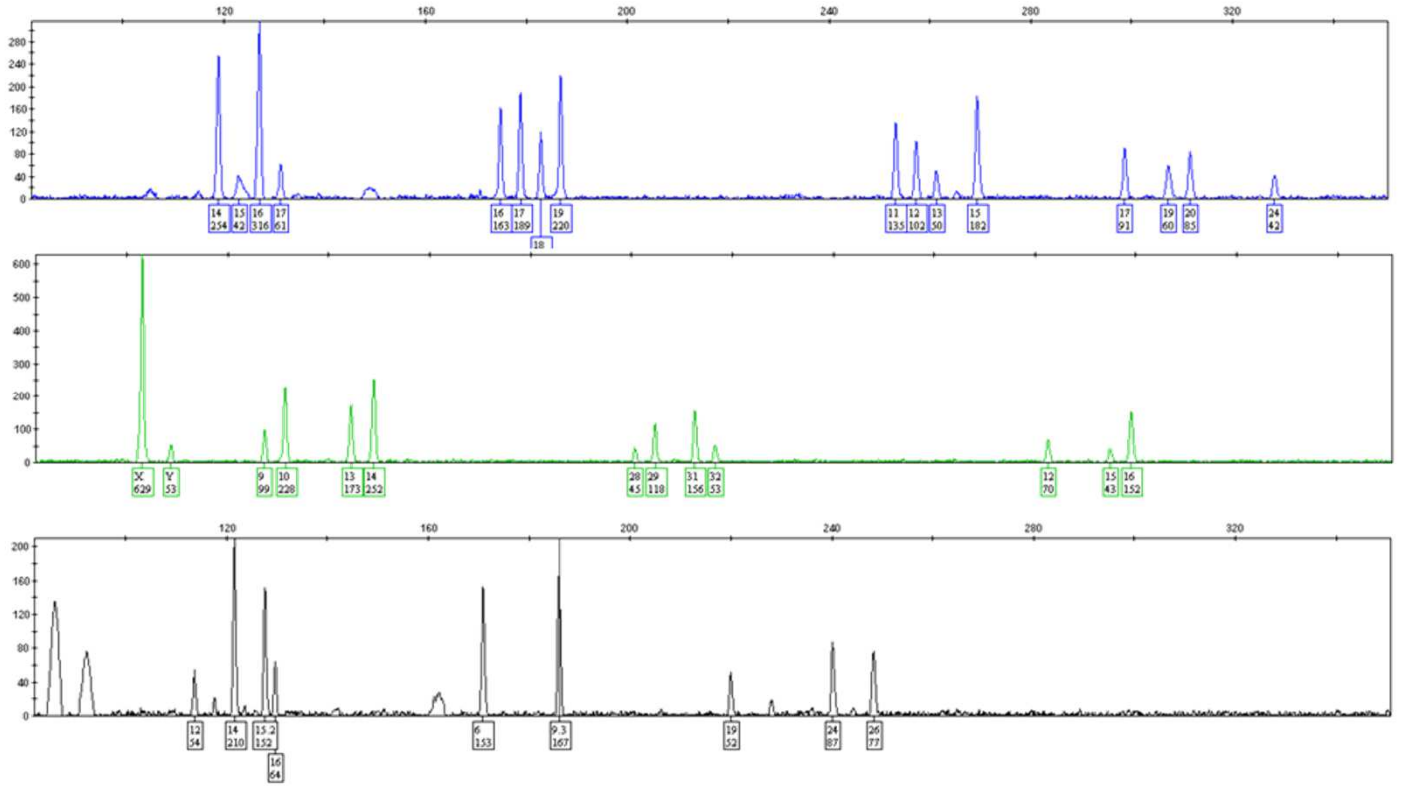
- The crime-stain is from an epithelial swab taken from the female victim
- There are two suspects accused of sexual assault, S_1 and S_2 respectively; both deny the offence.
- This epg is classified as a low template of three or more individuals since there are multiple alleles per locus that fall within 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.

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

EPG



EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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).

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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*

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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.

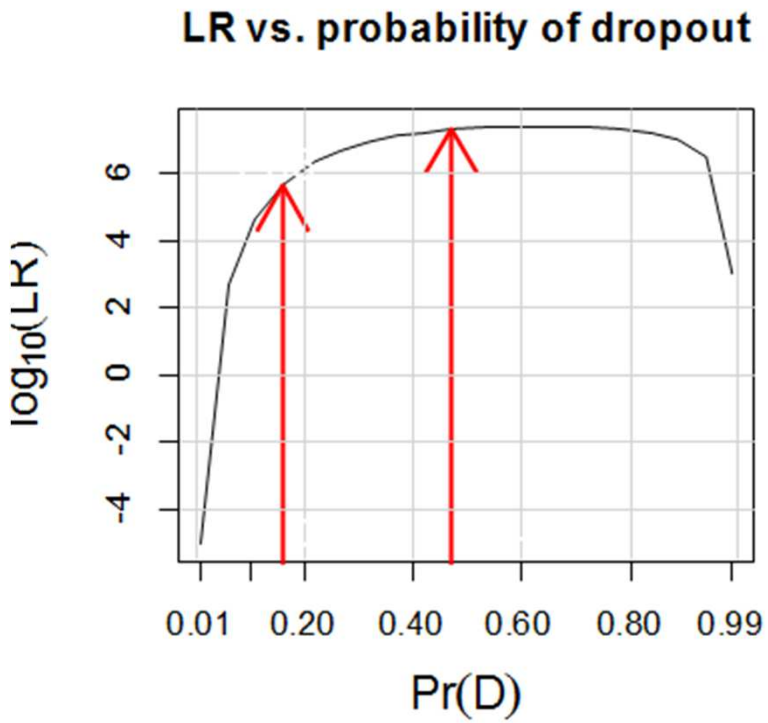
- Pr(D) value is in fact the 5 percentile calculated from an empirical distribution of the drop-out probability conditioned on the expected number of alleles observed relative to the genotype of the hypothesised contributors, the procedure is described by Haned et al (FISG 2012)

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Sensitivity plot



EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

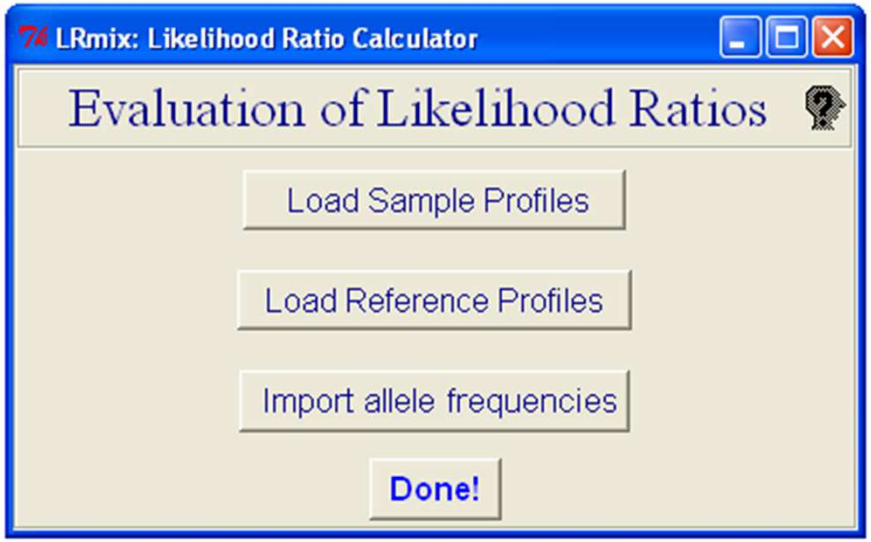
Main LRmix interface

Now we show how to:

(1)Load the crime-sample profile

(2)Load the references (suspect/victim)

(3)Load your allele frequencies



EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

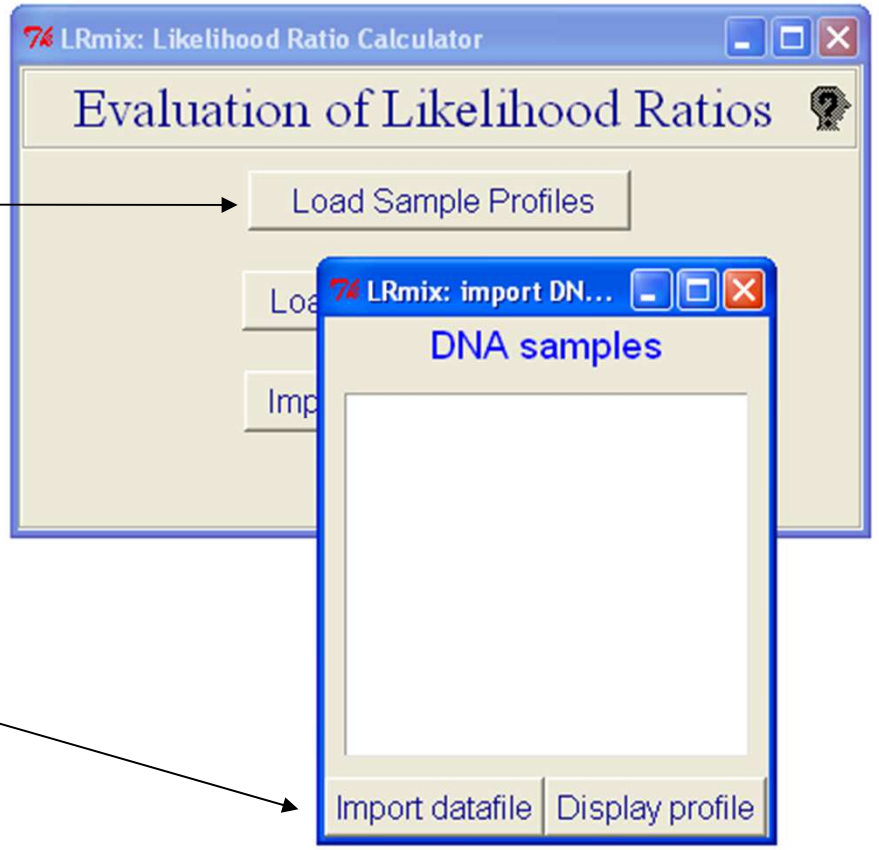
Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

(1) Load the crime-sample profiles

Click "Load Sample Profiles"

Click "Import datafile"



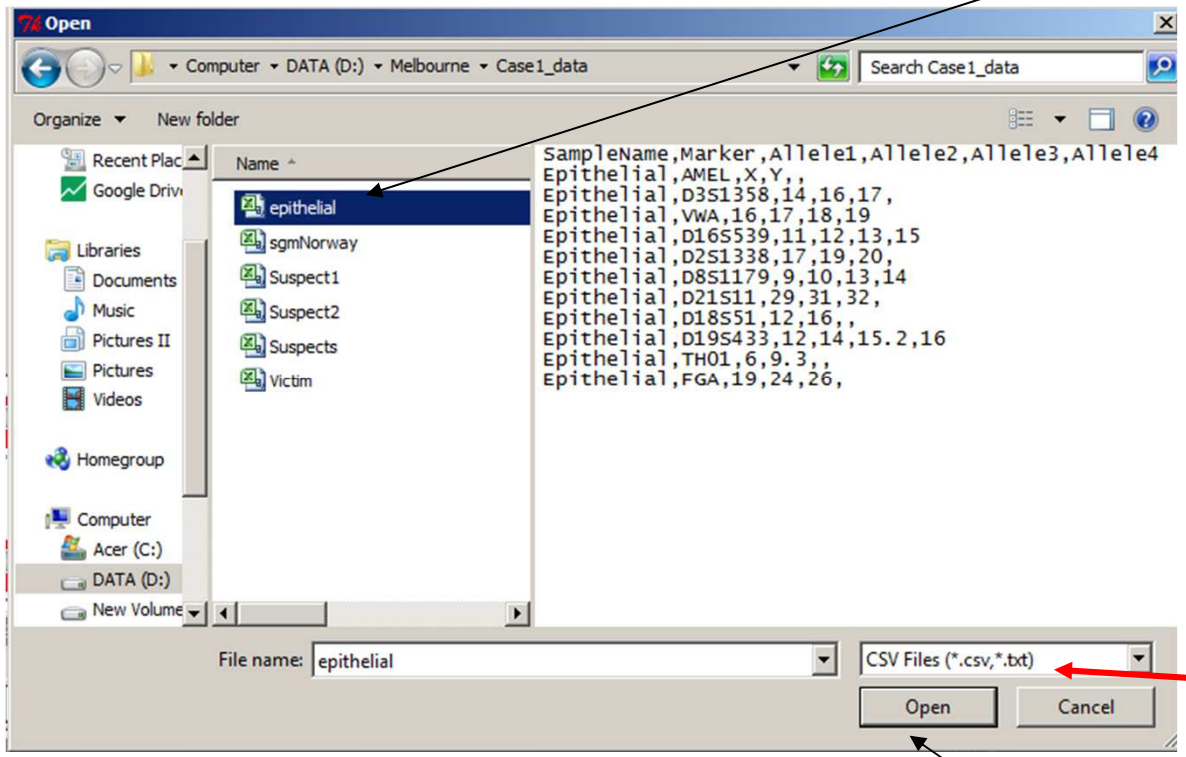
EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

(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

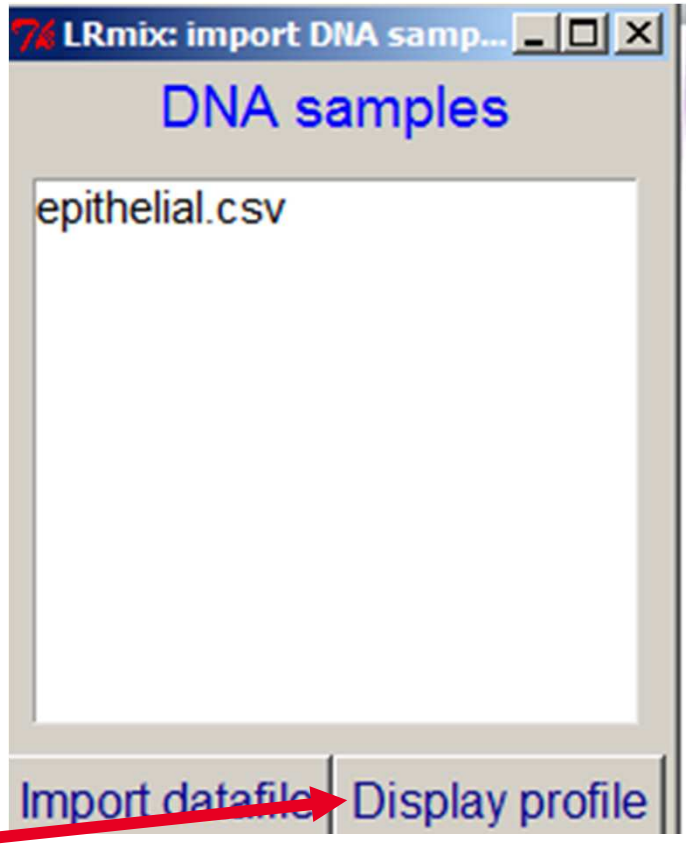
Then click 'Open'

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Display the crime-sample profile



Click ‘Display profile’,

To make sure the data is OK

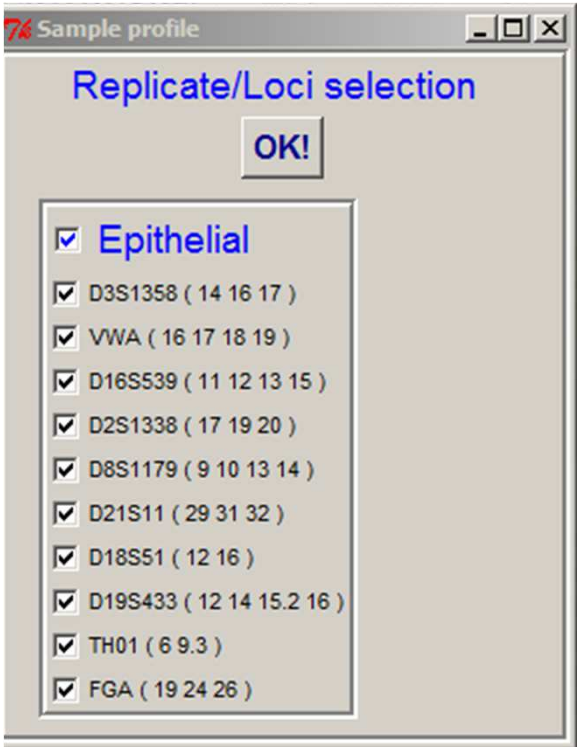
EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

If everything looks good, press OK!

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



EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

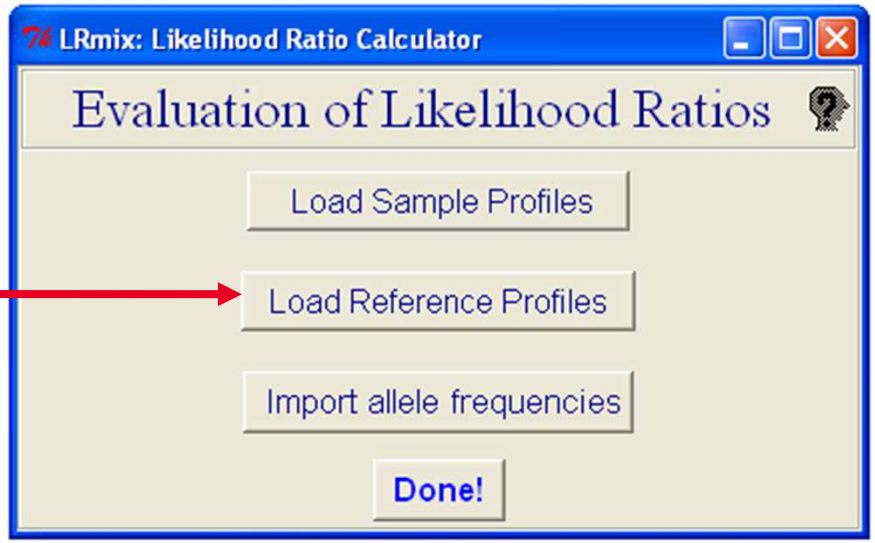
Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

(2) Load reference profiles from your folder

suspects

victim

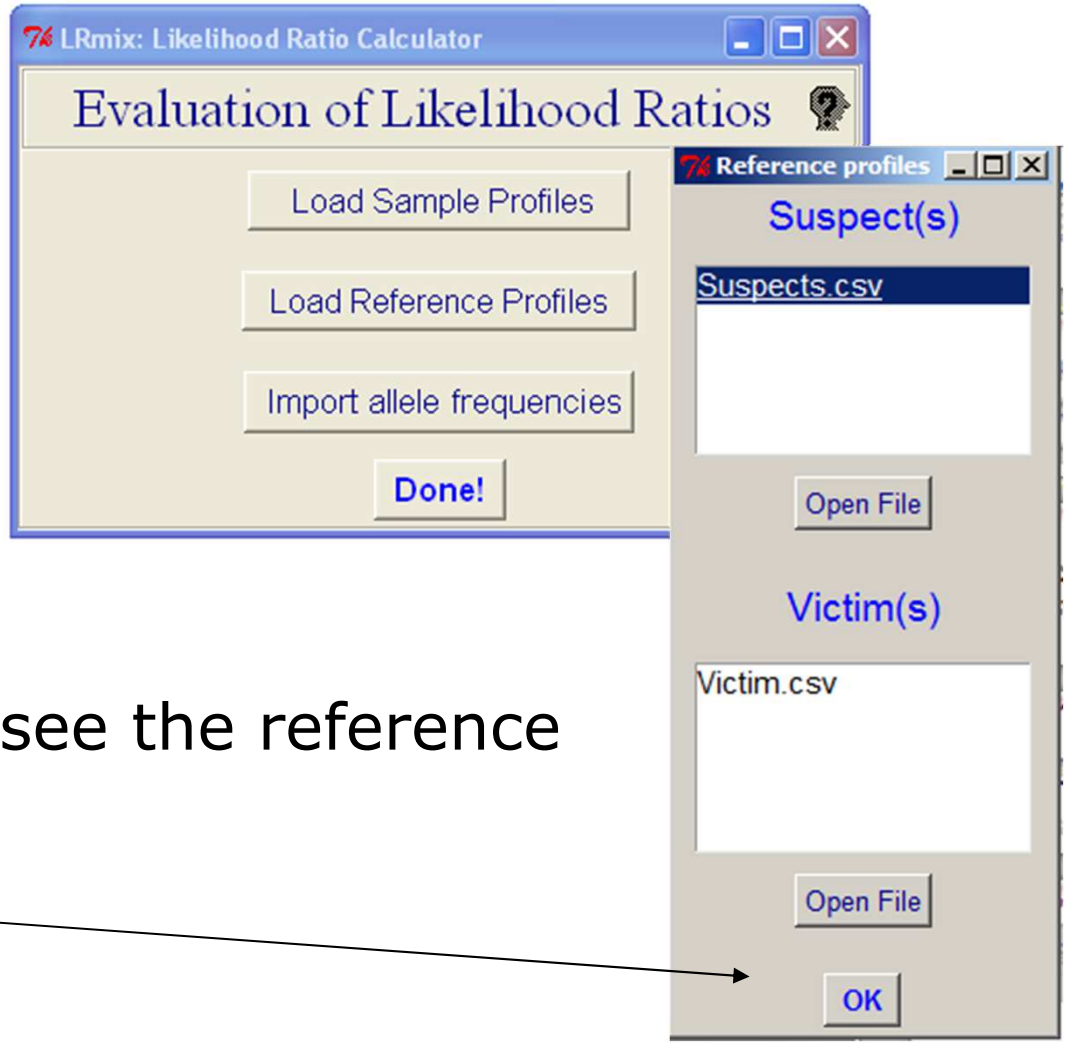


EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

(2) Load reference profiles from your folder



You cannot see the reference profiles

Press OK

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

- 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

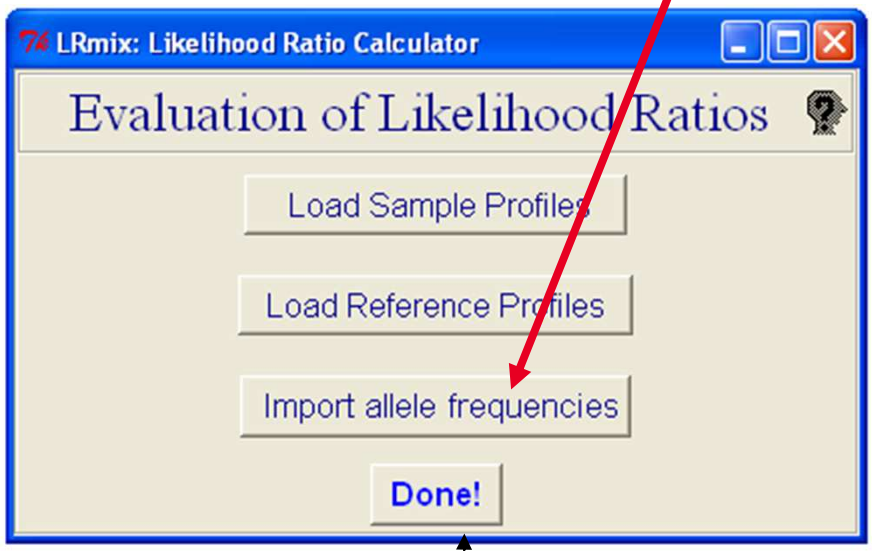
EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

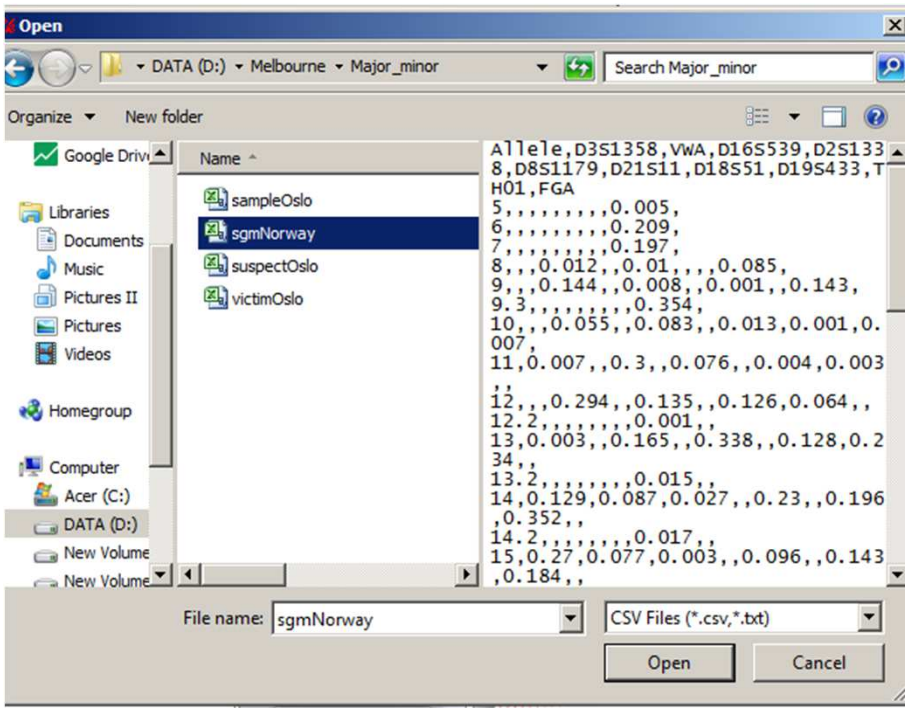
Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

(3) Import the allele frequencies

sgmNorway





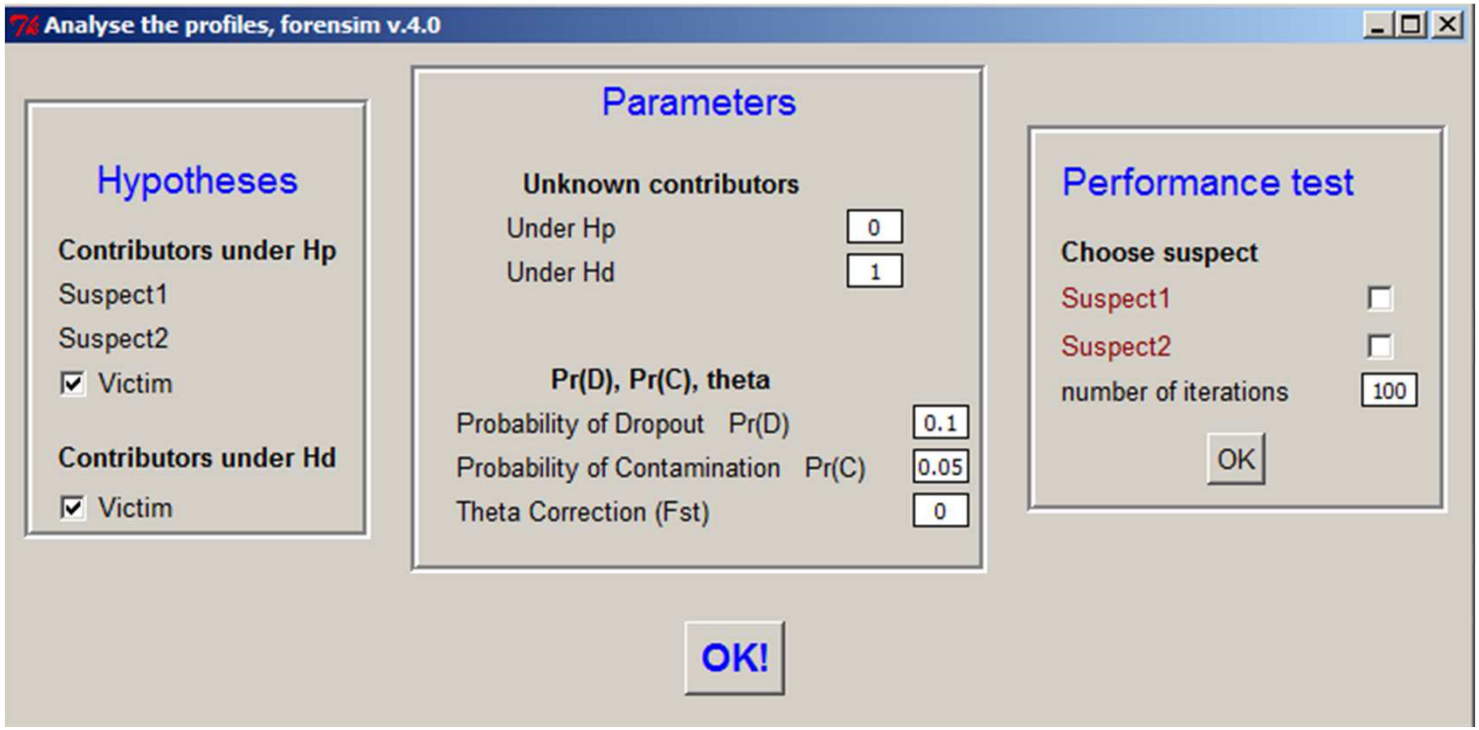
Once loaded, click ‘Done’

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Now you should see this



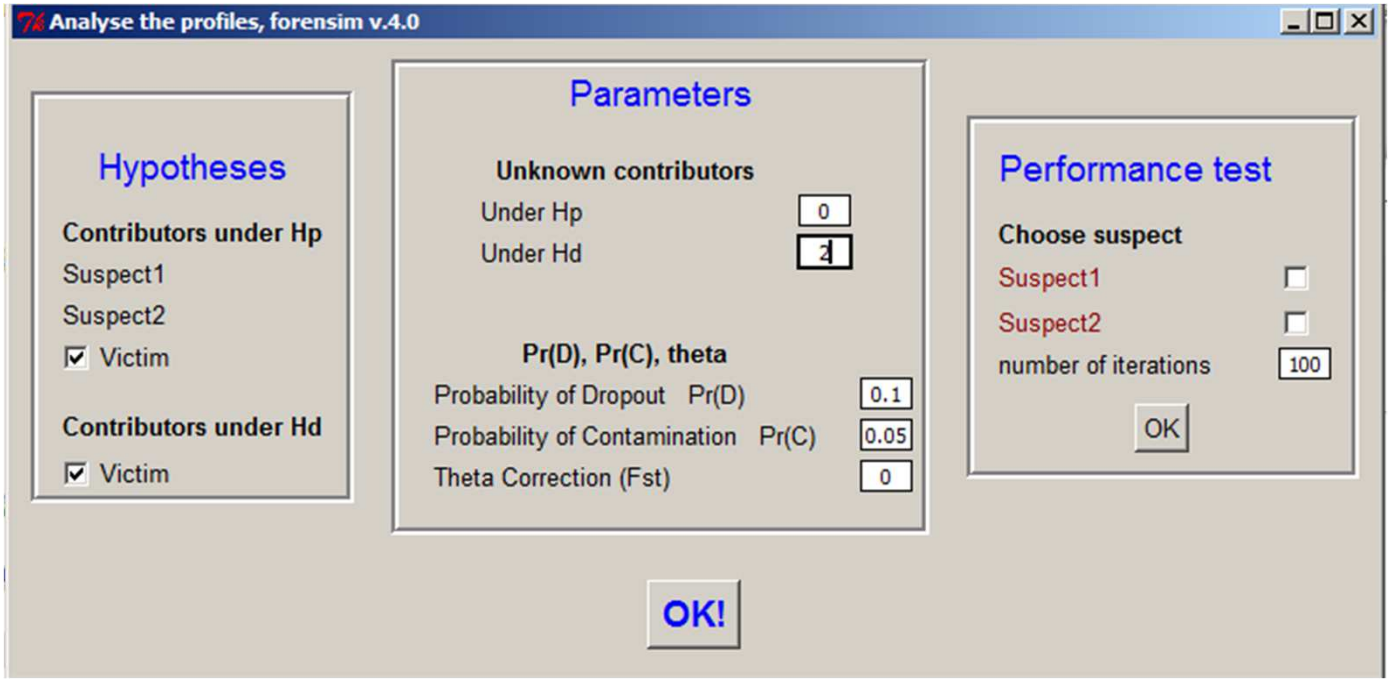
EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Alter the parameters

- 2 unknown contributors under Hd
- Click OK



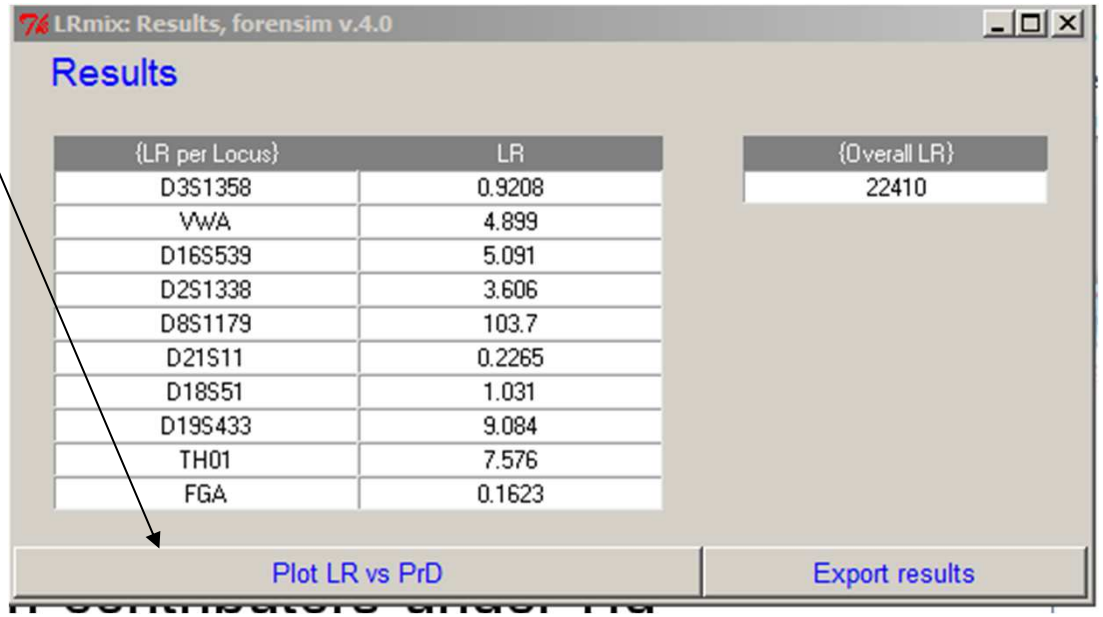
EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Results Table

- Carry out sensitivity analysis – click on button

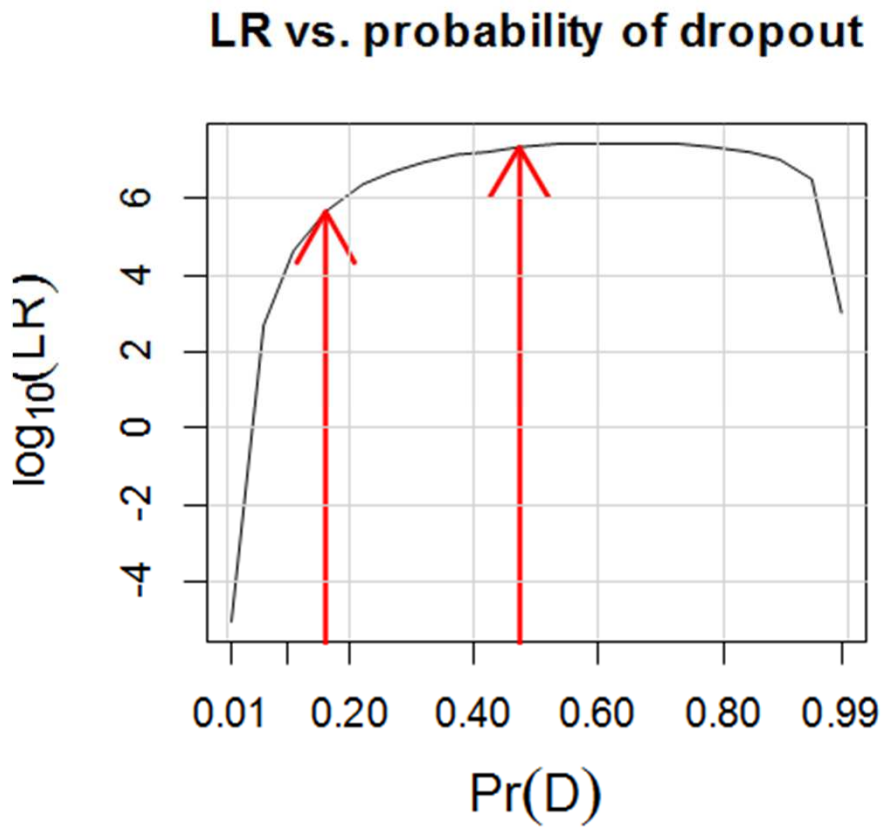


EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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 .

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

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?

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

Oskar Hansson

Statistical methods in forensic genetics 7-10 October 2013, Copenhagen

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

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Forensic Science International 160 (2006) 90–101

Forensic Science International

www.elsevier.com/locate/forsciint

DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

P. Gill [a,*], C.H. Brenner [b], J.S. Buckleton [c], A. Carracedo [d], M. Krawczak [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], P.M. Schneider [i], B.S. Weir [j]

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 1: The likelihood ratio is the preferred approach to mixture interpretation. The RMNE approach is restricted to DNA profiles where the profiles are unambiguous. If the DNA crime stain profile is low level and some minor alleles are the same size as stutters of major alleles, and/or if drop-out is possible, then the RMNE method may not be conservative.*

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 2: Even if the legal system does not implicitly appear to support the use of the likelihood ratio, it is recommended that the scientist is trained in the methodology and routinely uses it in case notes, advising the court in the preferred method before reporting the evidence in line with the court requirements. The scientific community has a responsibility to support improvement of standards of scientific reasoning in the court-room.*

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 3: The methods to calculate likelihood ratios of mixtures (not considering peak area) described by Evett et al [13] and Weir et al [14] are recommended.*

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 4: If peak height or area information is used to eliminate various genotypes from the unrestricted combinatorial method, this can be carried out by following a sequence of guidelines based on Clayton et al* [17].

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 5: The probability of the evidence under Hp is the province of the prosecution and the probability of the evidence under Hd is the province of the defence. The prosecution and defence both seek to maximise their respective probabilities of the evidence profile. To do this both Hp and Hd require propositions. There is no reason why multiple pairs of propositions may not be evaluated* (Appendix 3).

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Example of generalisation

- How many contributors in a DNA profile?
- Classically we decide on the number of contributors by counting the number of alleles present per locus
- By consideration of the casework circumstances

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

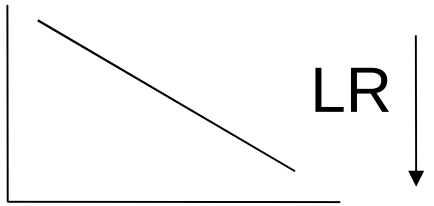
EUROFORGEN Network of Excellence

Anchoring the prosecution hypothesis

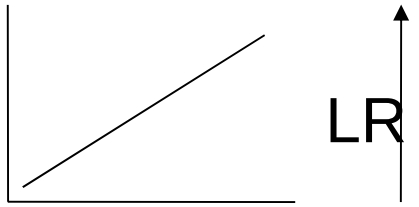
$$LR = \frac{\Pr E \mid Hp}{\Pr E \mid Hd}$$

Not anchored – the number of propositions is the same in numerator and denominator:

$$\frac{S + U_1 + U_2 + U_3}{U_0 + U_1 + U_2 + U_3}$$



Anchored - the number of propositions is different in numerator and denominator: $\frac{S + U_1}{U_0 + U_1 + U_2 + U_3}$



Contributors under Hd

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

How does this help?

- Usually the scientist decides the number of contributors on behalf of both prosecution and defence
- Minimising the number of contributors usually maximises the Probability on behalf of the defence
- The foregoing is a *generalisation* which may not always be true (Buckleton et al 2007).
- Is the generalisation true in this case?
- **check the *trend* by analysing multiple propositions**

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 6: If the crime-profile is a major/ minor mixture, where minor alleles are the same size (height or area) as stutters of major alleles, then stutters and minor alleles are indistinguishable. Under these circumstances alleles in stutter positions that do not support Hp should be included in the assessment.*

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 7: If drop-out of an allele is required to explain the evidence under Hp: (S = ab; E = a), then the allele should be small enough (height/area) to justify this (i.e. the allele should be below a predetermined threshold).*

- Basically, this means that if an allele found in the reference sample is missing in the crime stain then it is not necessarily neutral evidence.
- Reworking the sample is always important to see if we can recover the missing alleles.

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 8: When a DNA profile is at a level that is dominated by background noise, then a biostatistical interpretation should not be attempted.*

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

ISFG DNA commission recommendations

- *Recommendation 9: In relation to low copy number, stochastic effects limit the usefulness of heterozygous balance and mixture proportion estimates. In addition, allelic drop-out and allelic drop-in (contamination) should be taken into consideration of any assessment.*

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

New ISFG DNA commission

- New commission recently reported and recommends the incorporation of dropin and drop-out into probabilistic calculations

Forensic Science International: Genetics 6 (2012) 679–688

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

P. Gill [a,b,*], L. Gusmão [c], H. Haned [d], W.R. Mayr [e], N. Morling [f], W. Parson [g], L. Prieto [h], M. Prinz [i], H. Schneider [j], P.M. Schneider [k], B.S. Weir [l]

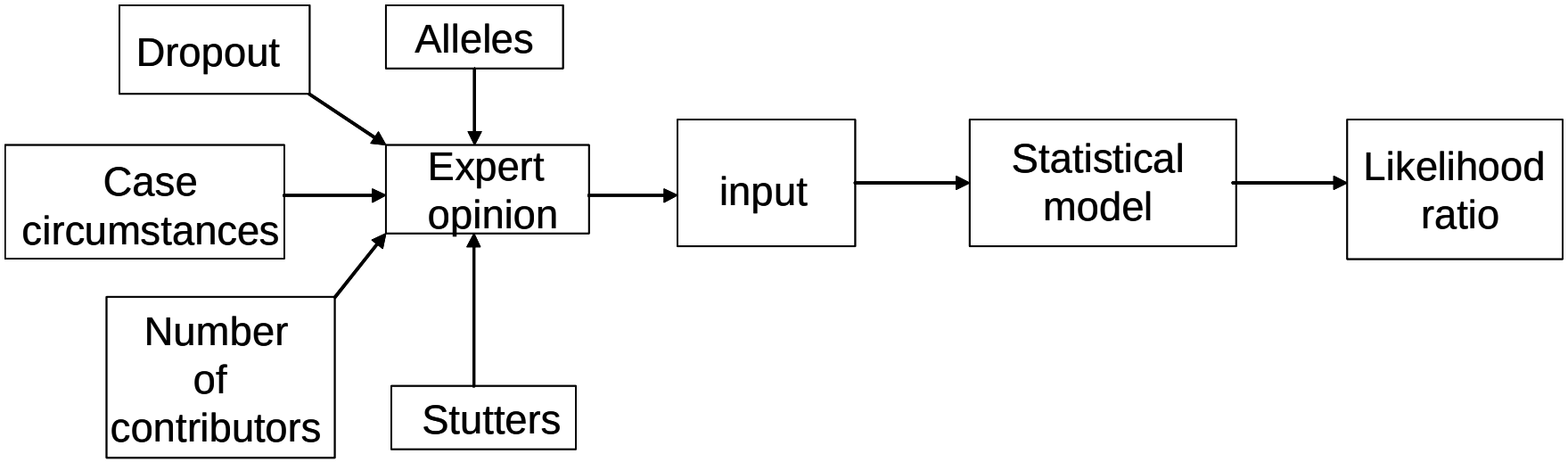
[a] Norwegian Institute of Public Health, Oslo, Norway
[b] University of Oslo, Oslo, Norway

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Interpretation process is an interaction of the expert with a statistical model



EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Numbers of contributors

- There is no need to anchor the number of contributors to be the same under Hp and Hd – they will often be different
- There will be differences between prosecution and defence hypotheses that courts will wish to explore. Software will facilitate the exploration

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

More generalisations

- Don’t ignore inconvenient (to the prosecution) events.
- Use statistical tools to explore the data so we can understand what is going on
- The statistical analysis may suggest that samples need to be reworked as a preferable option

EUROFORGEN-NoE is funded by the European Commission
within the 7th Framework Programme

Norwegian Institute of Public Health

EUROFORGEN Network of Excellence

Summary of New ISFG DNA commission recommendations

- Probabilistic methods following the *'basic model'* described here can be used to evaluate the evidential weight of DNA results considering drop-out and/or drop-in.
- Estimates of drop-out and drop-in probabilities should be based on validation studies that are representative of the method used.
- The weight of the evidence should be expressed following likelihood ratio principles.
- The use of appropriate software is highly recommended to avoid hand-calculation errors.

SEVENTH FRAMEWORK PROGRAMME

EUROFORGEN-NoE is funded by the European Commission within the 7th Framework Programme