



Software - FamLinkX

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GHEP-OS Spring 2025

● WORKSHOP 1

X-chromosomal markers in forensic genetics
Daniel Kling & Andreas Tillmar

● WORKSHOP 2

Acreditación en el campo de la Genética Forense y estrategias de validación de ensayos
Manuel Crespillo Márquez, Rosalía Izquierdo & Estel Enreig Cabanes

● WORKSHOP 3

La genética en la Identificación de víctimas a gran escala: comparación de perfiles y evaluación estadística con Familias
Carlos Vullo & Lourdes Prieto

Teachers

Daniel Kling, PhD



- Forensic Expert
- National Board of Forensic Medicine, Sweden
- Worked in the field for almost 15 years
- Developer of Familias, FamLink and FamLinkX
- Applied biostatistics, relationship inference, genetic genealogy

Andreas Tillmar, PhD



- Forensic geneticist & Associate professor
- National Board of Forensic Medicine, Sweden and Linköping University, Sweden
- Worked in the field for almost 20 years
- Technical leadership mixed with R&D
- Applied biostatistics, relationship inference, population genetics, genetic genealogy



Disclaimer!

Points of view are those of the presenters and do not necessarily represent the official position or policies of the National Board of Forensic Medicine or ISFG. Certain commercial software, instruments, and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement, nor does it imply that any of the materials, instruments, or equipment identified are necessarily the best available for the purpose.

Topics

- Software
- FamLinkX – Demonstration
- Advanced topics
 - Creating pedigrees
 - Advanced settings
 - Simulations
- Validation



SOFTWARE

Software

➤ Several publications on formulas

Legal Medicine
Volume 32, May 2018, Pages 9-18

A simple method for calculating the likelihood ratio in a kinship test using X-chromosomal markers incorporating linkage, linkage disequilibrium, and mutation

Mamiko Fukuta ^a, ^b, Mohammed Hassan Gaballah, Hideaki Kato, Yasuhiro Aoki

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Received 12 July 2017, Revised 30 January 2018, Accepted 9 February 2018, Available online 12 February 2018, Version of Record 20 March 2018.

What do these dates mean?

Check for updates

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<https://doi.org/10.1016/j.legalmed.2018.02.002> Get rights and content

Highlights

- Japanese population data of 27 X-chromosomal short tandem repeats were collected.
- A simple method for calculating the likelihood ratio in a kinship test is provided.
- A simulation study showed high discrimination ability of the calculation method.

Forensic Science International: Genetics
Volume 6, Issue 2, March 2012, Pages 198-207

A general method to assess the utility of the X-chromosomal markers in kinship testing

Nádia Pinto ^a, ^b, ^c, Pedro V. Silva ^b, ^c, António Amorim ^a, ^b

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<https://doi.org/10.1016/j.fsigen.2011.04.014> Get rights and content

Abstract

In studies involving pedigree reconstruction and kinship estimation, it is acknowledged that some pedigrees have the same algebraic expressions for the joint genotypic probabilities and are, therefore, indistinguishable when considering only genetic information, no matter what the mode of transmission considered. Indeed, although standard forensic practice considers solely unlinked autosomal markers, the existence of pedigrees with the referred theoretical property (that are then said to belong to the same kinship class) is possible when considering any kind of genetic transmission. The research on genetic relatedness has always been linked to the root concept of identity-by-descent (IBD). However, although the basic theoretical core for autosomal transmission has been long formalised, a general method allowing the decision if two pedigrees linking two non-inbred individuals are distinguishable using unlinked

Forensic Science International: Genetics
Volume 5, Issue 1, January 2011, Pages 27-32

X-chromosome markers in kinship testing: A generalisation of the IBD approach identifying situations where their contribution is crucial

Nádia Pinto ^a, ^b, ^c, Leonor Gusmão ^a, António Amorim ^a, ^b

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<https://doi.org/10.1016/j.fsigen.2010.01.011> Get rights and content

Abstract


The standard practice of forensic kinship evaluation uses unlinked autosomal markers. However, X-chromosome markers have recently gained recognition as a powerful tool to complement the information provided by autosomes, particularly in complex cases.

Software

➤ Several publications on formulas



Forensic Science International: Genetics
Volume 5, Issue 1, January 2011, Pages 27-32



X-chromosome markers in kinship testing: A generalisation of the IBD approach identifying situations where their contribution is crucial

Nádia Pinto ^{a b c}, Leonor Gusmão ^a, António Amorim ^{a b}

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<https://doi.org/10.1016/j.fsigen.2010.01.011> [Get rights and content](#)

Abstract

The standard practice of forensic kinship evaluation uses unlinked autosomal markers. However, X-chromosome markers have recently gained recognition as a powerful tool to complement the information provided by **autosomes**, particularly in complex cases.

Box 1. Joint genotypic probabilities for X-markers and two females (Example)


Consider two commonly tested kinships involving two females where the analysis of X-markers seems to be useful: paternal half-sisters and paternal aunt – niece, assuming non-inbred females. Below we present the IBD probabilities associated with these pedigrees, as well as with unrelated X-chromosomal transmission.

Paternal half-sisters	Paternal uncle-niece	Unrelated
		
$x_0^{FF} = 0$ $x_1^{FF} = 1$ $x_2^{FF} = 0$	$x_0^{FF} = 1/2$ $x_1^{FF} = 1/2$ $x_2^{FF} = 0$	$x_0^{FF} = 1$ $x_1^{FF} = 0$ $x_2^{FF} = 0$

Once the IBD probabilities are established we just have to substitute those parameters into the formulas of Table 1 to obtain the joint genotypic probabilities:

Genotypes	joint genotypic probabilities for X-markers and non-inbred females		
	Paternal half-sisters	Paternal aunt-niece	Unrelated
A_Ai, A_Ai	f_i^3	$1/2 f_i^3 + 1/2 f_i^4$	f_i^4
A_Ai, A_Aj	0	$1/2 f_i^2 f_j^2$	$f_i^2 f_j^2$
A_Ai, A_Ai	$f_i^2 f_j$	$1/2 f_i^2 f_j + f_i^3 f_j$	$2 f_i^3 f_j$
A_Aj, A_Ai	$f_i^2 f_j$	$1/2 f_i^2 f_j + f_i^3 f_j$	$2 f_i^3 f_j$
A_Ai, A_Ak	0	$f_i^2 f_k$	$2 f_i^2 f_k$
A_Ak, A_Ai	0	$f_i^2 f_k$	$2 f_i^2 f_k$
A_Ai, A_Aj	$f_i f_j (f_i + f_j)$	$1/2 f_i f_j (f_i + f_j) + 2 f_i^2 f_j^2$	$4 f_i^2 f_j^2$
A_Ai, A_Ak	$f_i f_k$	$1/2 f_i f_k + 2 f_i^2 f_k$	$4 f_i^2 f_k$
A_Aj, A_Aj	0	$2 f_j f_k f_l$	$4 f_j f_k f_l$

Software



ChrX-STR.org 2.0

Database and information hub for forensic X-chromosomal markers

Home Content

Software and Kits

Marker Haplotypes Evaluate & Calculate Submit Data

Software and Kits

Literature


Links

Manage

Manage access

News

Based on the review of december 2018, it has been decided in cooperation with the X working group to remove the PI calculation from this website.



The software FamLinkX provides functions for likelihood calculation on family relationships/pedigrees using linked DNA marker data located on the ChrX. FamLinkX is a freely available software, accessible via <http://www.FamLink.se>. The software was developed by Daniel Kling, Andreas Tillmar, Thore Egeland and Petter Mostad [1, 2].

The statistical tool FamLinkX can be used for the interpretation of clusters of linked markers located on the X chromosome. It requires haplotype frequencies and can model mutations and recombinations within a cluster. The main function is to calculate case specific likelihood ratios (LR) with observed DNA-data for X-chromosomal markers. The software provides an easy-to-use graphical user interface for Windows systems.

[1] Kling D, Tillmar A, Egeland T, Mostad P. A general model for likelihood computations of genetic marker data accounting for linkage, linkage disequilibrium, and mutations. *Int J Legal Med.* 2015 Sep;129(5):943-54.

[2] Kling D, Dell'Amico B, Tillmar AO. FamLinkX - implementation of a general model for likelihood computations for X-chromosomal marker data. *Forensic Sci Int Genet.* 2015 Jul;17:1-7.

GenoProof® Suite

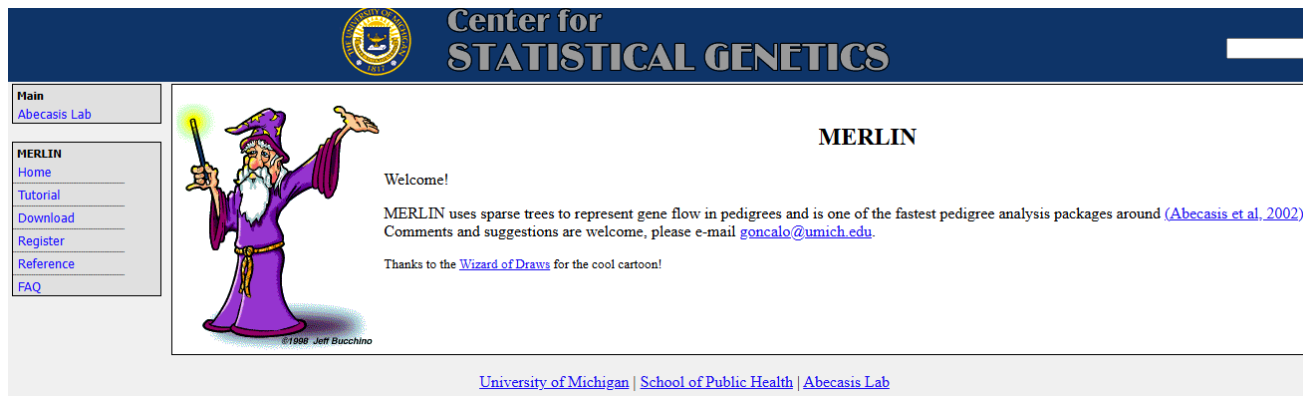
GenoProof® Suite is a professional all-in-one solution for DNA analysis in the field of forensic molecular genetics. The application allows you to put together an individual solution tailored to your needs by selecting special expert modules and to expand it later if required.

The kinship module supports the entire kinship investigation process, from genotyping samples to performing biostatistical calculations and generating reports.

The software determines all important parameters for standard trio and duo constellations and even for unusual scenarios like

Software

Merlin



The screenshot shows the homepage of the MERLIN software. At the top is a dark blue header with the University of Michigan logo and the text "Center for STATISTICAL GENETICS". Below the header is a navigation menu on the left with links for "Main", "Abecasis Lab", "MERLIN", "Home", "Tutorial", "Download", "Register", "Reference", and "FAQ". The main content area features a cartoon wizard holding a glowing wand, with the text "MERLIN" in large letters. Below the wizard, there is a "Welcome!" message, a paragraph explaining that MERLIN uses sparse trees for gene flow in pedigrees and is one of the fastest pedigree analysis packages, and a thank-you note to the "Wizard of Draw" for the cartoon. At the bottom of the page, there are links for "University of Michigan", "School of Public Health", and "Abecasis Lab".

MINX: Chromosome X Analyses

MINX (MERLIN in X) is an X-specific version of Merlin. It is available in distributions of MERLIN version 0.9.1 and later. There is currently no manuscript describing MINX performance and algorithms in detail. Although I believe MINX results to be correct, the methods are unpublished and I would advise using with care.

MINX implements X-chromosome specific versions of the functions provided by the standard Merlin implementation. Males are hemizogous and carry only one X chromosome.

```
D:\>merlin.exe -d markers.dat -m markers.map -f markers.freq -p pedigree.ped --lik --perFamily > results.txt
```

Software

GenoProof

The screenshot shows the qualitype website. At the top left is the qualitype logo, and at the top right are navigation links for HOME and PR. Below the navigation is a breadcrumb trail: HOME > PRODUCTS > GENOPROOF SUITE > MODULE KINSHIP. The main content area features a large orange banner with the text "GenoProof® Suite Expert Module Kinship" over a background of a network diagram. Below the banner is a white box containing the "GenoProof® Suite" logo and a sub-heading "Kinship - Your expert module for relationship analysis". A short paragraph follows, describing the module's capabilities in explaining kinship relationships and mapping complex pedigrees.

qualitype

HOME PR

HOME > PRODUCTS > GENOPROOF SUITE > MODULE KINSHIP

GenoProof® Suite Expert Module Kinship

GenoProof® Suite

Kinship - Your expert module for relationship analysis

The expert module Kinship offers you a wide range of possibilities to explain kinship relationships biostatistically and to map complex pedigrees - from ancestry and output of pedigrees to forensic biostatistics with identity probabilities and contamination control.

Software

FamLinkX



ELSEVIER

Forensic Science International: Genetics

Volume 17, July 2015, Pages 1-7



FamLinkX – implementation of a general model for likelihood computations for X-chromosomal marker data

[Daniel Kling](#)^{a, b}  , [Barbara Dell'Amico](#)^c , [Andreas O. Tillmar](#)^{c, d} 

Show more 

FamLinkX

- Windows software (can be run on Macs etc)
- Written in C++
- Works on any marker kit for the X-chromosome
- Developed for the Argus X12 kit (But works just as well with Decaplex or SNPs!)
- Any relationships (e.g. several typed)
- Has a **single** parameter that the user needs to set

FamLinkX

➤ Algorithm

Home > [International Journal of Legal Medicine](#) > Article

A general model for likelihood computations of genetic marker data accounting for linkage, linkage disequilibrium, and mutations

Original Article | Published: 26 November 2014
Volume 129, pages 943–954, (2015) [Cite this article](#)

[Download PDF](#) Access provided by National Library of Sweden (Kungliga Biblioteket)

[Daniel Kling](#) , [Andreas Tillmar](#), [Thore Egeland](#) & [Petter Mostad](#)

1088 Accesses 24 Citations 6 Altmetric [Explore all metrics](#) →

Abstract

Several applications necessitate an unbiased determination of relatedness, be it in linkage or association studies or in a forensic setting. An appropriate model to compute the joint probability of some genetic data for a set of persons given some hypothesis about the pedigree structure is then required. The increasing number of markers available through high-density SNP microarray typing and NGS technologies intensifies the demand, where using a large number of markers may lead to biased results due to strong dependencies between closely located loci, both within pedigrees (linkage) and in the population (allelic association or linkage disequilibrium (LD)). We present a new general model, based on a Markov chain for inheritance patterns and another Markov chain for founder allele patterns, the latter allowing us to account for LD. We also demonstrate a specific implementation for X chromosomal markers that allows for computation of likelihoods based on hypotheses of alleged relationships and genetic marker data. The algorithm can simultaneously account for linkage, LD, and mutations. We demonstrate its feasibility using simulated examples. The algorithm is implemented in the software FamLinkX,

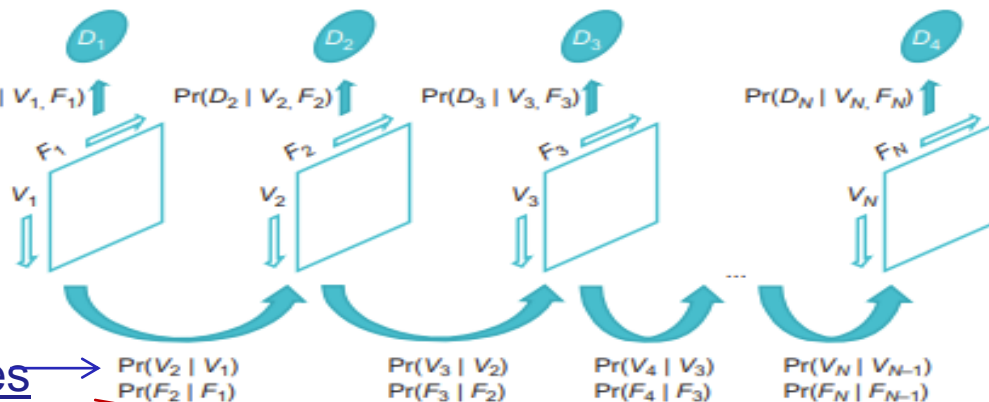


FamLinkX

➤ Algorithm

$$\Pr(\text{data} | H) = \sum_{V_1} \dots \sum_{V_N} \sum_{F_1} \dots \sum_{F_N} \Pr(V_1) \Pr(F_1) \prod_{i=2}^N \Pr(V_i | V_{i-1}) \prod_{i=2}^N \Pr(F_i | F_{i-L}, \dots, F_{i-1}) \prod_{i=1}^N \Pr(D_i | V_i, F_i), \quad (4.9)$$

Pedigree likelihood



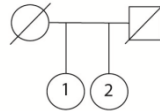
Recombination rates

Haplotype frequencies

FamLinkX

Follows some basic steps (similar to most forensic genetic software)

1. Define frequency data (and other parameters)
 - a) Alleles and frequencies
 - b) Haplotypes
 - c) Mutation parameters (rates etc)
 - d) Advanced settings
2. Specify pedigree (hypotheses)
 - a) Main hypothesis (e.g. Full siblings)
 - b) Alternative hypotheses
3. Define case-specific DNA data (STR or SNP data)
4. Compute LR (Different methods)



FamLinkX

- Define frequency database (Haplotypes if necessary)

Database name:

Cluster	Chromosome	Number of markers	Number of haplotyp
Cluster1	X	3	367
Cluster2	X	3	220
Cluster3	X	3	195
Cluster4	X	3	301

Cluster

Add

Edit

Remove

Import

Export

Options

Close

Argus X12

FamLinkX

- Define frequency database (Haplotypes if necessary)

Database name:

Cluster	Chromosome	Number of markers	Number of haplotyp
DXS8378	X	1	0
DXS9902	X	1	0
DXS7132	X	1	0
DXS9898	X	1	0
DXS6809	X	1	0
DXS6809	X	1	0
DXS7133	X	1	0
GATA172D05	X	1	0
GATA31E08	X	1	0
DXS7423	X	1	0

Cluster

Add

Edit

Remove

Import

Export

Options

Close

Decaplex

FamLinkX

- Define frequency database (Haplotypes if necessary)

Edit cluster: Cluster1

Allele systems

System	Number of al...	Position (cM)
DXS10148	22	10.000000
DXS10135	30	11.123000
DXS8378	6	11.263000

Actions

Add

Edit

Remove

Import

Export

Observed haplotypes

Name	Counts	DXS10148	DXS10135
1	1	13.3	33.2
2	2	14	21
3	1	14	22
4	1	14	26
5	1	14	32
6	1	17	27
7	2	18	18
8	2	18	18
9	1	18	18
10	1	18	18
11	1	18	18.1
12	1	18	19
13	2	18	19
14	2	18	19

Actions

Add

Edit

Remove

Remove all

Lambda

1

Estimate frequency

General

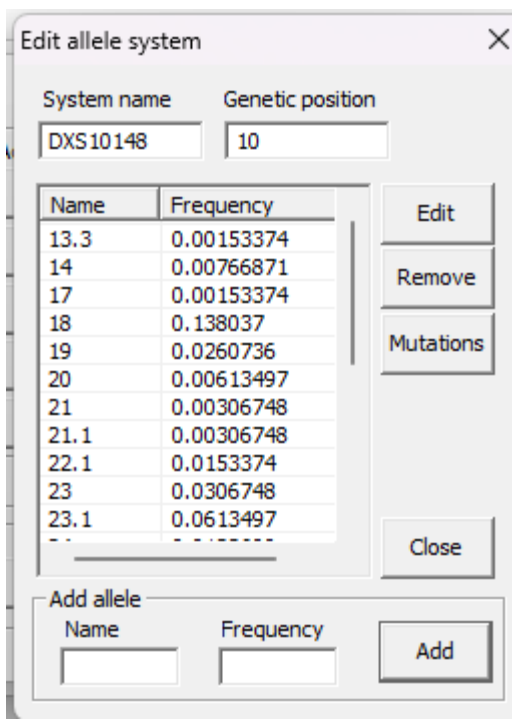
Cluster name: Cluster1

Chromosome: X

Close

FamLinkX

- Define frequency database (Haplotypes if necessary)



Dialog box titled "Edit allele system" with a close button (X).

System name: Genetic position:

Name	Frequency
13.3	0.00153374
14	0.00766871
17	0.00153374
18	0.138037
19	0.0260736
20	0.00613497
21	0.00306748
21.1	0.00306748
22.1	0.0153374
23	0.0306748
23.1	0.0613497

Buttons: Edit, Remove, Mutations, Close

Add allele section:

Name	Frequency	Add
<input type="text"/>	<input type="text"/>	<input type="button" value="Add"/>

FamLinkX

➤ Frequency data import format

Allele frequency matrix

ALLELES	DXS10148	DXS10135	DXS8378	DXS7132
7	0,00000	0,00000	0,00000	0,00000
8	0,00000	0,00000	0,00641	0,00000
9	0,00000	0,00000	0,00641	0,00000
10	0,00000	0,00000	0,29487	0,00870
11	0,00000	0,00000	0,36538	0,03478
12	0,00000	0,00000	0,28205	0,12174
13	0,00000	0,00000	0,04487	0,26087
13.3	0,00641	0,00000	0,00000	0,00000
14	0,00000	0,00000	0,00000	0,31304
15	0,00000	0,00641	0,00000	0,18261

FamLinkX

➤ Frequency data import format

Allele frequency

```
DXS8378
10      0.425925925925926
11      0.296296296296296
12      0.259259259259259
13      0.0185185185185185
```

```
DXS9898
8.3     0.0740740740740741
10      0.0185185185185185
11      0.0555555555555556
12      0.333333333333333
13      0.407407407407407
14      0.111111111111111
```

```
DXS7133
9        0.5
10      0.222222222222222
11      0.203703703703704
12      0.0740740740740741
```

FamLinkX

➤ Frequency data import format

Haplotypes

Haplotype	DXS10148	DXS10135	DXS8378	Count
Germa1	13.3	28	12.0	1
Germa2	13.3	29	12.0	1
Germa3	14	27	12.0	1
Germa4	16	22.1	10.0	1
Germa5	16.1	27	10.0	1
Germa6	17	27	12.0	1
Germa7	18	18	11.0	1
Germa8	18	27	11.0	1
Germa9	18	27	11.0	1
Germa10	18	27	11.0	1
Germa11	18	27	12.0	1

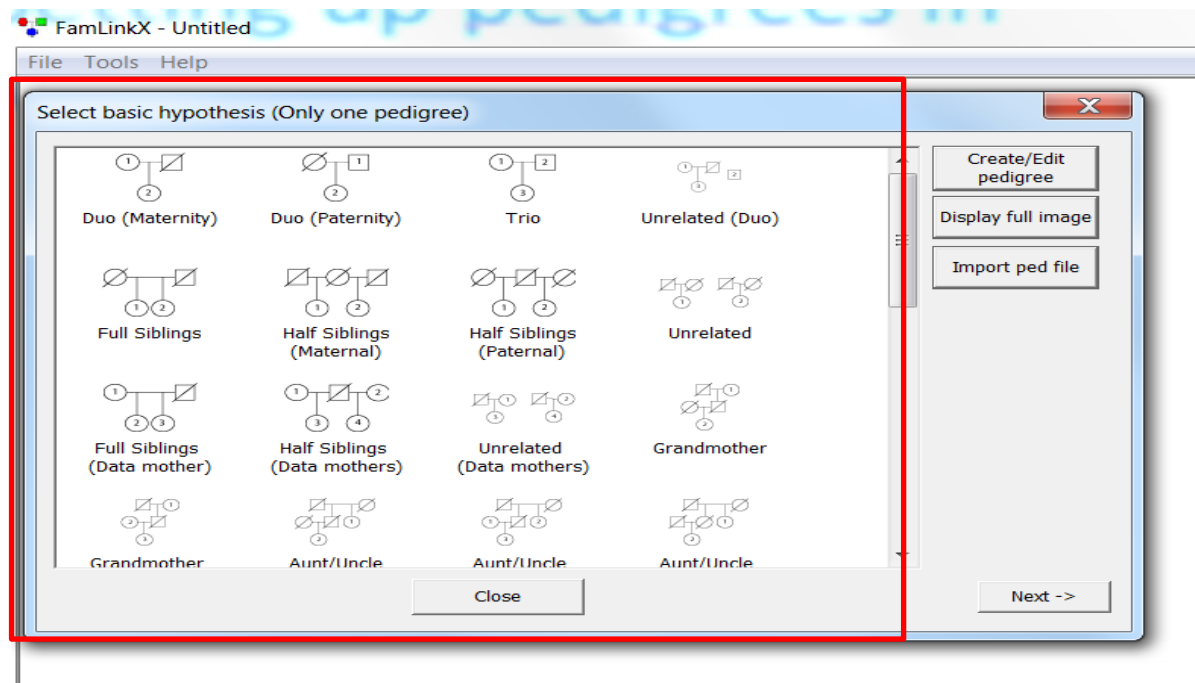
FamLinkX

➤ Specify hypotheses

Standard set of
pedigrees

Total of 50

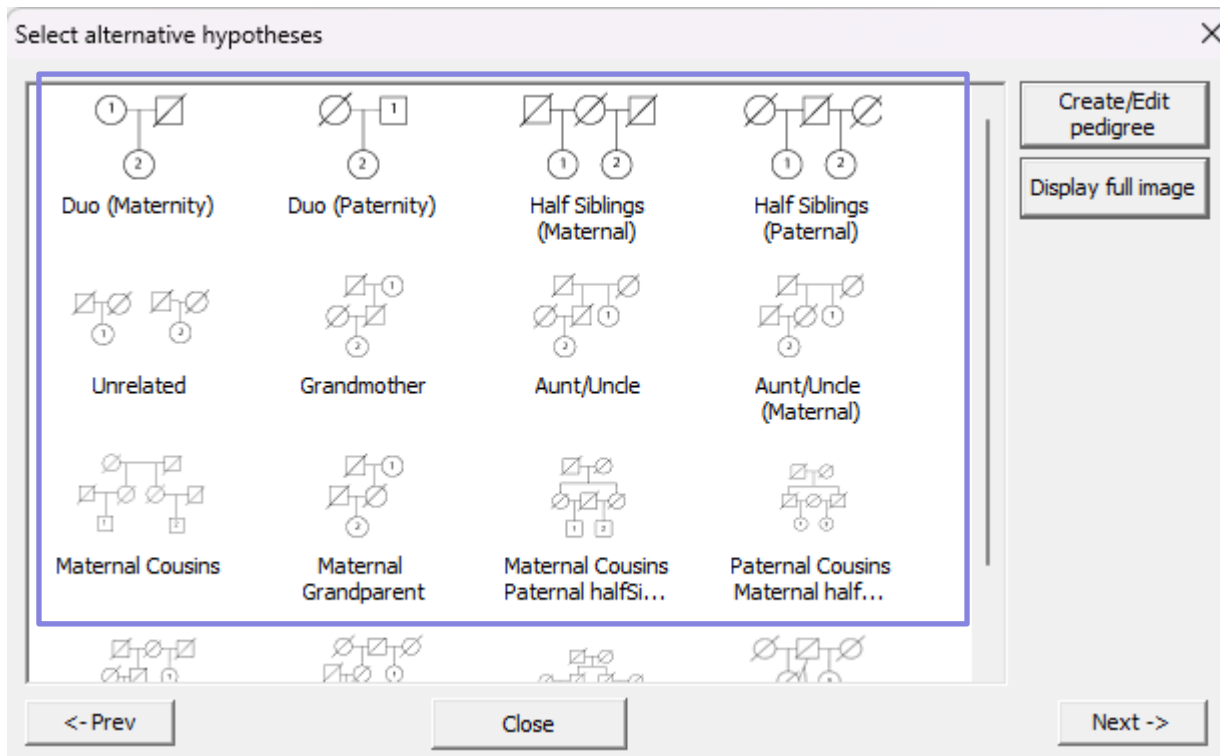
Full model



FamLinkX

➤ Specify hypotheses

Two typed individuals



FamLinkX

➤ Specify hypotheses

Three typed individuals

Select alternative hypotheses

Trio

Unrelated (Duo)

Full Siblings (Data mother)

Grandmother (Data mother)

Aunt/Uncle (Data mother)

Two Aunts/Uncles

Two Full Siblings One Half Sibling

Two Full Siblings One Paternal ...

Two Full Siblings One Unrelated

Mother and child Unrelated person

Half siblings (Maternal) ...

Half siblings (Paternal) ...

Create/Edit pedigree

Display full image

<- Prev

Close

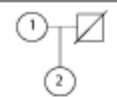
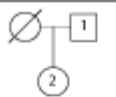
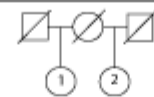
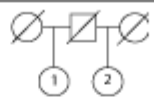


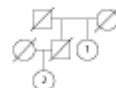



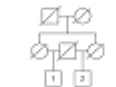





Next ->

FamLinkX

➤ Specify hypotheses

Genders can be changed in most pedigrees!

Select alternative hypotheses

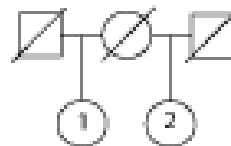
 Duo (Maternity)	 Duo (Paternity)	 Half Siblings (Maternal)	 Half Siblings (Paternal)
 Unrelated	 Grandmother	 Aunt/Uncle	 Aunt/Uncle (Maternal)
 Maternal Cousins	 Maternal Grandparent	 Maternal Cousins Paternal halfSi...	 Paternal Cousins Maternal half...
			

<- Prev Close

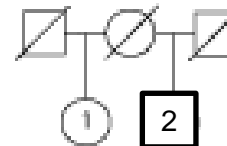
FamLinkX

➤ Specify hypotheses

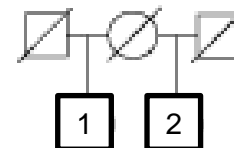
Genders can be changed in most pedigrees!



Half Siblings
(Maternal)



Half Siblings
(Maternal)



Half Siblings
(Maternal)

FamLinkX

➤ Specify hypotheses

Genders can be changed in most pedigrees!

Not in all!

Select alternative hypotheses

The screenshot shows a window titled "Select alternative hypotheses" with a grid of 16 pedigree diagrams. Each diagram is labeled with a hypothesis name. The "Duo (Paternity)" hypothesis, which shows a female (circle with a slash) and a male (square with a vertical line) connected by a horizontal line, with a vertical line leading down to a female (circle with a slash) labeled "2", is highlighted with a red rectangular box. Other hypotheses include "Duo (Maternity)", "Half Siblings (Maternal)", "Half Siblings (Paternal)", "Unrelated", "Grandmother", "Aunt/Unde", "Aunt/Unde (Maternal)", "Maternal Cousins", "Maternal Grandparent", "Maternal Cousins Paternal halfSi...", "Paternal Cousins Maternal half...", and several partially visible options at the bottom.

<- Prev

Close

FamLinkX

➤ Import case-related DNA data

Add DNA data

Basic hypothesis [Half siblings (Paternal) (Data mother)]

1 ——— 2 ——— 3

2. NN

Name: NN

Gender: Male Female

Cluster: -

Marker: []

Alleles: [] []

DNA data

Cluster 1
DXS10148: 19, 25.1
DXS10135: 20.1, 24
DXS8378: 10, 12

Cluster 2
DXS7132: 12, 15
DXS10079: 19, 20
DXS10074: 8, 16.3

Cluster 3
DXS10103: 18, 20
HPRTB: 11, 14
DXS10101: 30.2, 31

Cluster 4
DXS10148: 24, 31

Compare data

<- Prev Close Import data Next ->

Main hypothesis

Individuals DNA data

FamLinkX

➤ Import case-related DNA data

Edit DNA data

2. NN

Name

NN

Gender

Male Female

Cluster

Cluster1

Marker

DXS10148

Alleles

19 25.1

DNA data

Cluster 1

DXS10148: 19, 25.1
DXS10135: 20.1, 24
DXS8378: 10, 12

Cluster 2

DXS7132: 12, 15
DXS10079: 19, 20
DXS10074: 8, 16.3

Cluster 3

DXS10103: 18, 20
HPRTB: 11, 14
DXS10101: 30.2, 31

Cluster 4

DXS10146: 24, 31

Compare data

Import data

Next ->

Manually edit DNA data

FamLinkX

➤ Import format for case-specific DNA data

”Familias-like” format

Sample name:	Ameloger	Ameloger	DXS10148	DXS10148	DXS10135	DXS10135	DXS8378 1	DXS8378
Mother	X	X	22.1	27.1	24	25	10	1
Child	X	X	25.1	27.1	24	25	11	1

xml (CODIS-like) format

Genemapper-like format

FamLinkX

➤ Import format for case-specific DNA data

”Familias-like” format

xml (CODIS-like) format

Genemapper-like format

```
<?xml version="1.0" encoding="UTF-8"?>
<CODISImportFile xmlns="urn:CODISImportFile-schema">
  <SPECIMEN>
    <SPECIMENID>Mother</SPECIMENID>
    <SPECIMENCATEGORY>Mother</SPECIMENCATEGORY>
    <SPECIMENNATIONALITY>Europe</SPECIMENNATIONALITY>
    <LOCUS>
      <LOCUSNAME>AmeIogenin</LOCUSNAME>
      <ALLELE>
        <ALLELEVALUE>X</ALLELEVALUE>
        <ALLELEHEIGHT>2958</ALLELEHEIGHT>
      </ALLELE>
      <ALLELE>
        <ALLELEVALUE>X</ALLELEVALUE>
      </ALLELE>
    </LOCUS>
    <LOCUS>
      <LOCUSNAME>DXS10103</LOCUSNAME>
      <ALLELE>
        <ALLELEVALUE>16</ALLELEVALUE>
        <ALLELEHEIGHT>866</ALLELEHEIGHT>
      </ALLELE>
      <ALLELE>
        <ALLELEVALUE>19</ALLELEVALUE>
        <ALLELEHEIGHT>750</ALLELEHEIGHT>
      </ALLELE>
    </LOCUS>
    <LOCUS>
      <LOCUSNAME>DXS8378</LOCUSNAME>
      <ALLELE>
        <ALLELEVALUE>11</ALLELEVALUE>
        <ALLELEHEIGHT>1368</ALLELEHEIGHT>
      </ALLELE>
      <ALLELE>
        <ALLELEVALUE>12</ALLELEVALUE>
        <ALLELEHEIGHT>1274</ALLELEHEIGHT>
      </ALLELE>
    </LOCUS>
  </SPECIMEN>
</CODISImportFile>
```


FamLinkX

➤ Import format for case-specific DNA data

”Familias-like” format

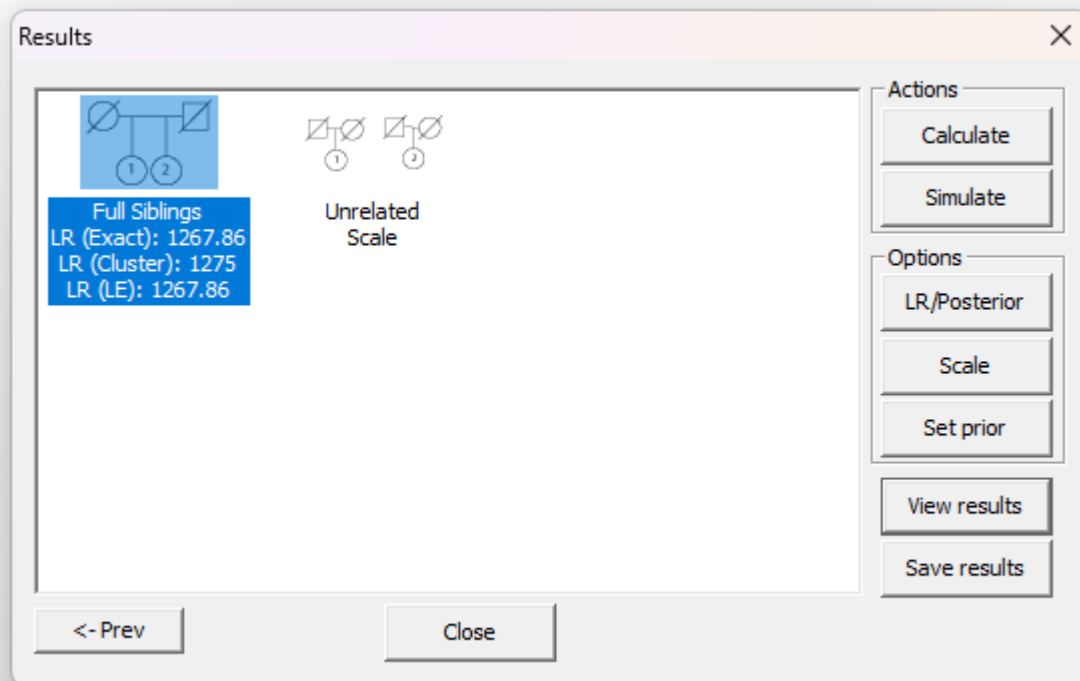
xml (CODIS-like) format

Genemapper-like format

Sample	Marker	Allele 1	Allele 2
Mother	DXS10135	14	16
Mother	DXS7123	20	23

FamLinkX

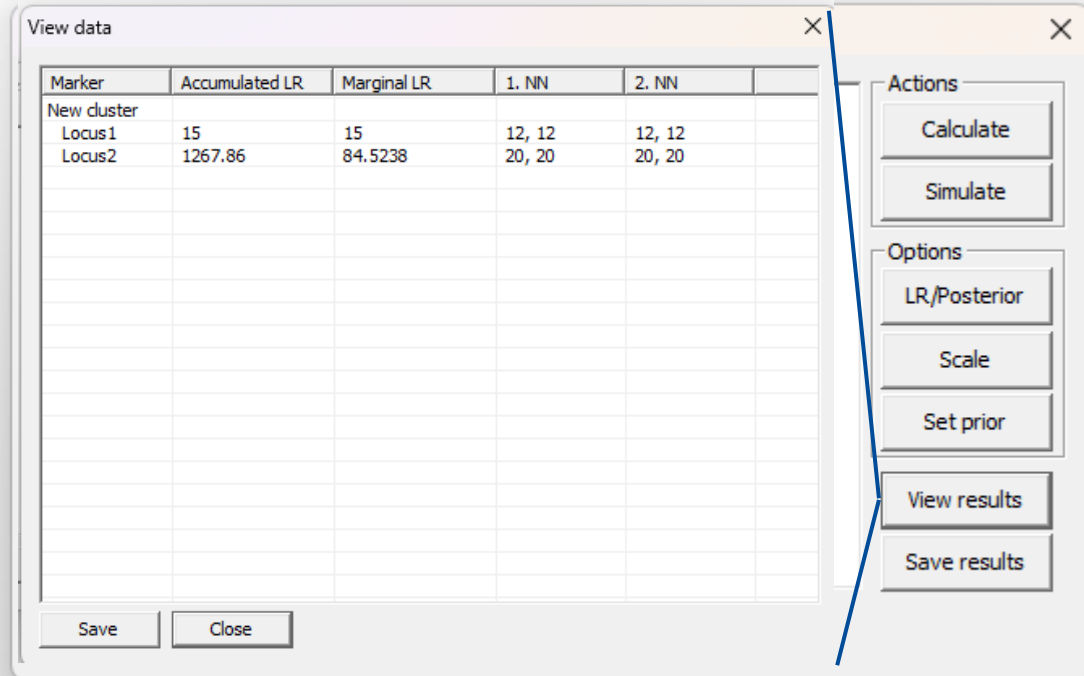
➤ Calculate LR



Three different methods to compute LR (more later)

FamLinkX

➤ Calculate LR



Marker	Accumulated LR	Marginal LR	1. NN	2. NN
New cluster				
Locus1	15	15	12, 12	12, 12
Locus2	1267.86	84.5238	20, 20	20, 20

Buttons: Calculate, Simulate, LR/Posterior, Scale, Set prior, View results, Save results, Save, Close

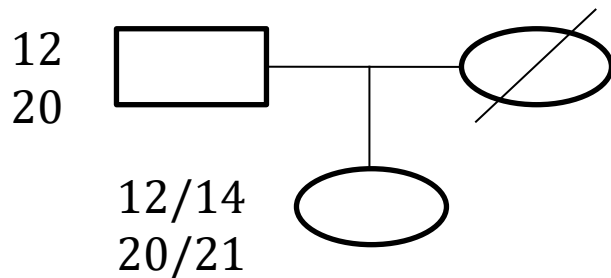
Individual marker LRs



FamLinkX – Basics

DEMONSTRATION 1

Demonstration



$$LR = 1/2p_{12} * 1/2p_{20} = 5 * 5 = \underline{25}$$

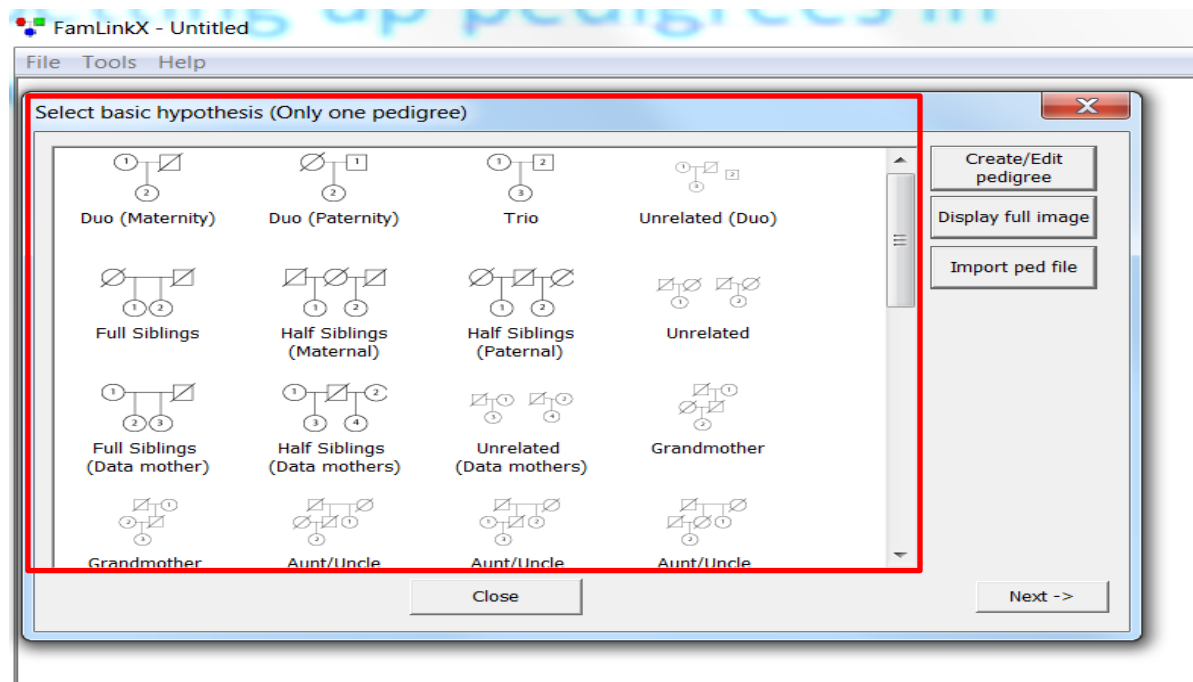
Marker	Position (cM)	Alleles	Frequencies
DXS10148	19.84	12, 14	0.1, 0.9
DXS10135	20.03	20, 21	0.1, 0.9



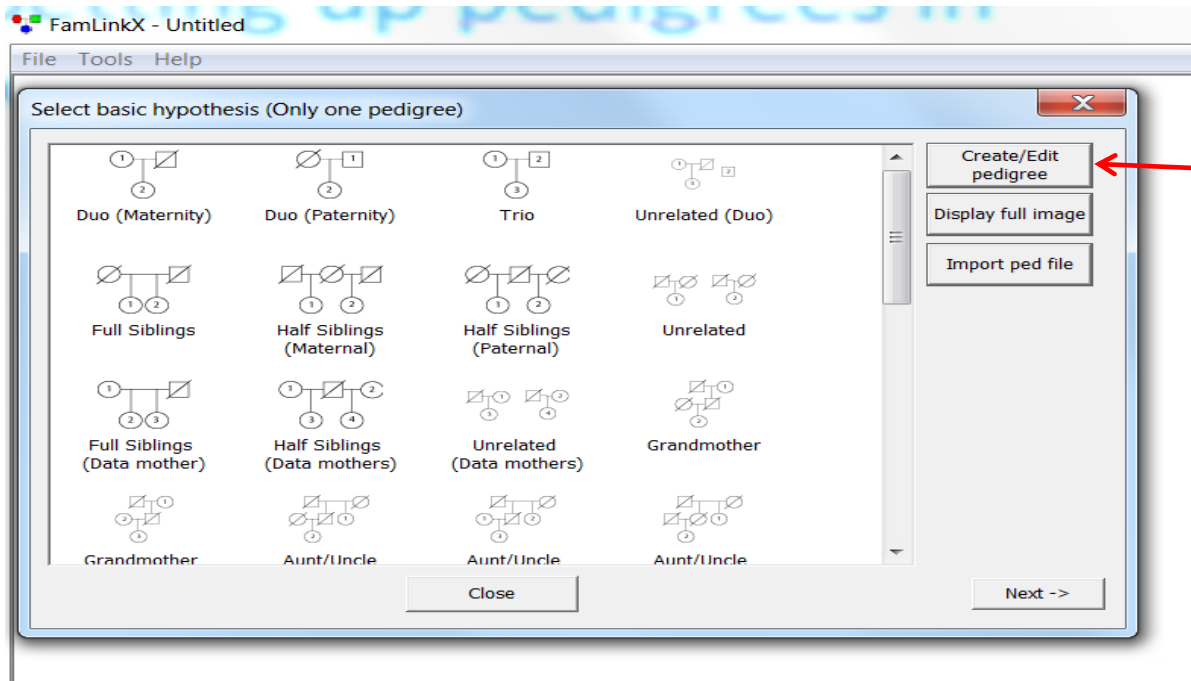
ADVANCED TOPICS

Creating pedigrees in FamLinkX

Standard set of pedigrees

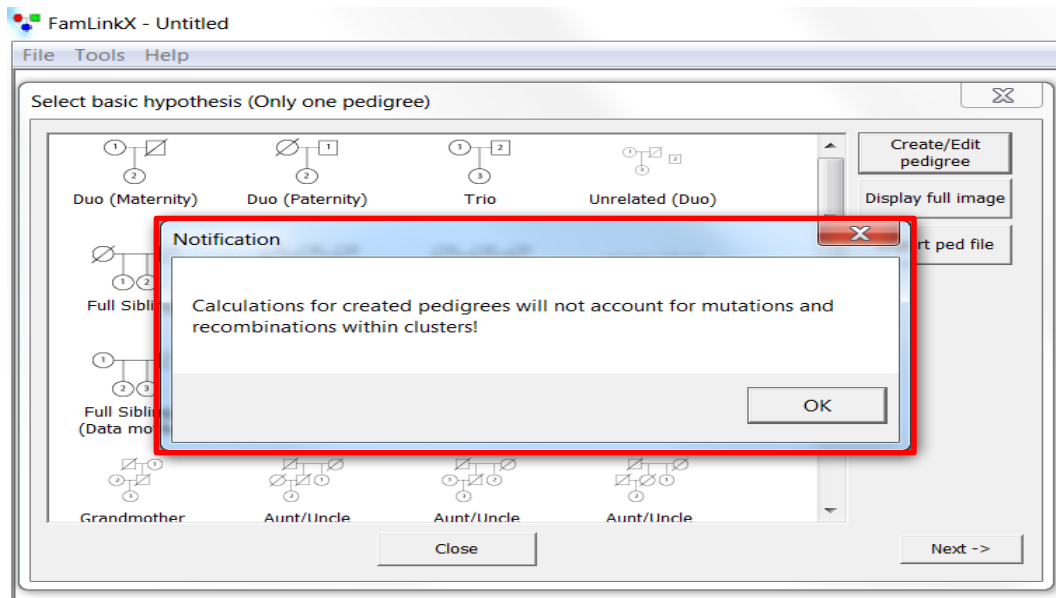


Creating pedigrees in FamLinkX



→ Create your own!

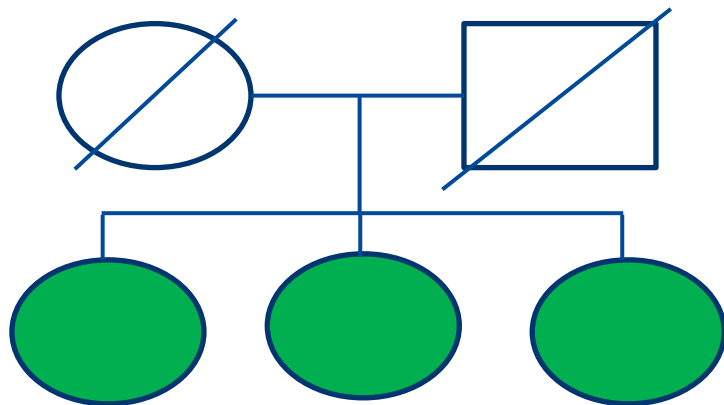
Creating pedigrees in FamLinkX



Created pedigrees will use Merlin for computations and mutations will not be considered!

Creating pedigrees in FamLinkX

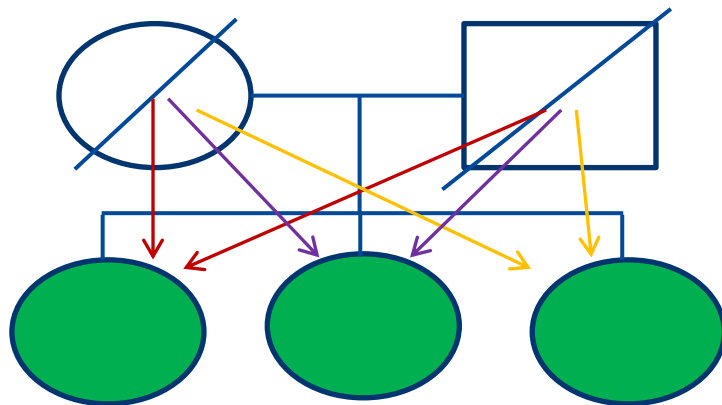
We first need to define the persons involved



2 untyped parents
3 typed children

Creating pedigrees in FamLinkX

Next identify relations



6 relations in total

Creating pedigrees in FamLinkX

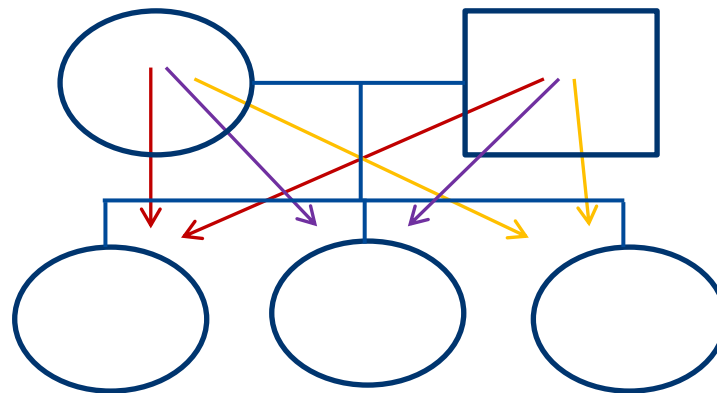
General recipe

1. Identify persons

2. Identify the parents of each person

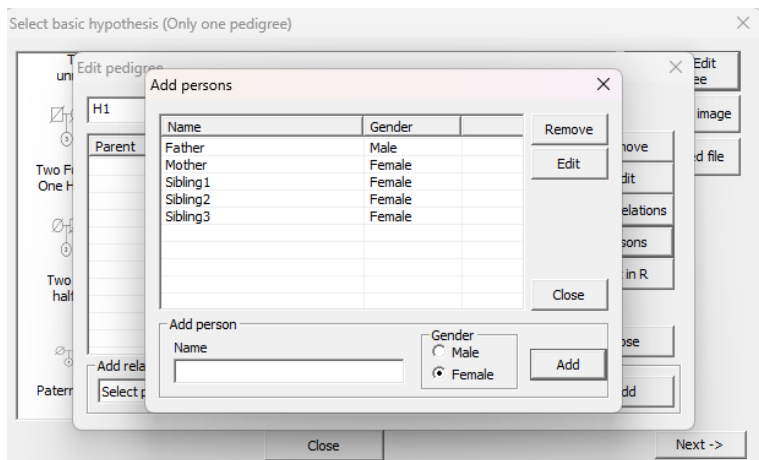
3. Known relations

4. Pedigree specific relations

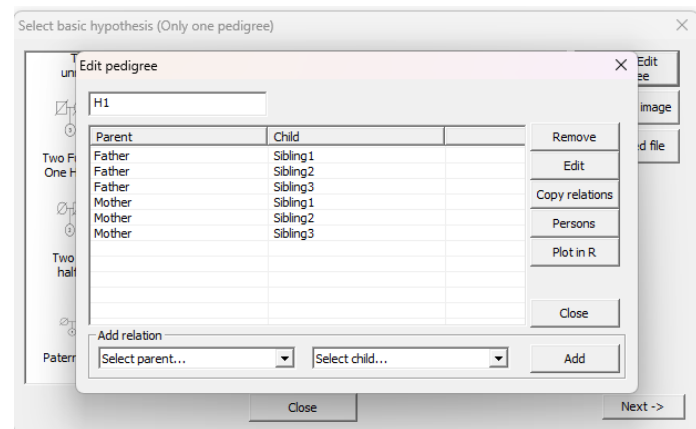


Six relations

Creating pedigrees in FamLinkX

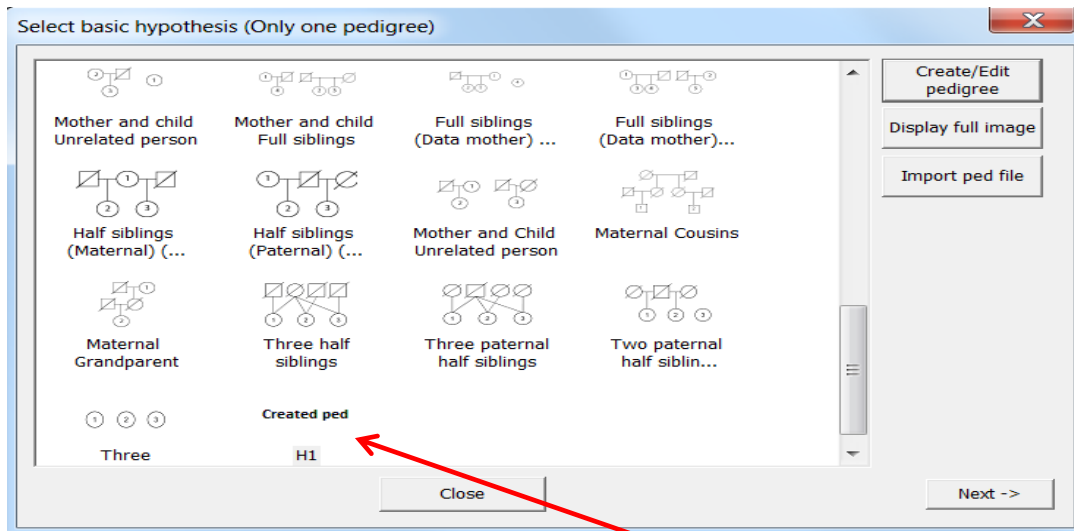


Persons



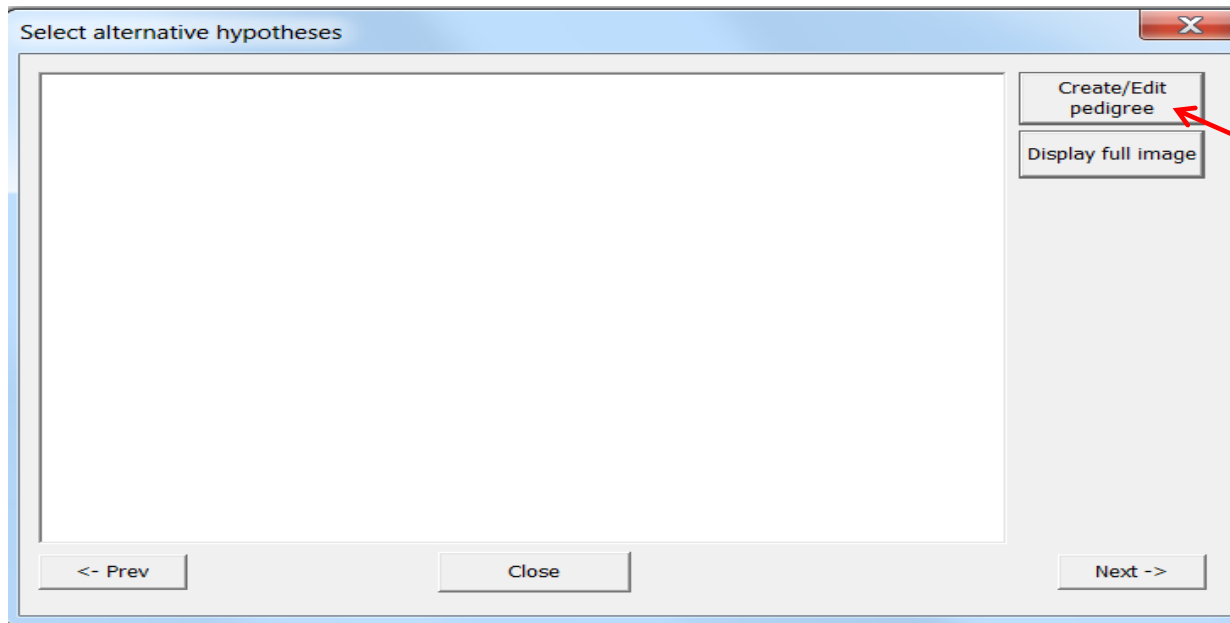
Relations

Creating pedigrees in FamLinkX



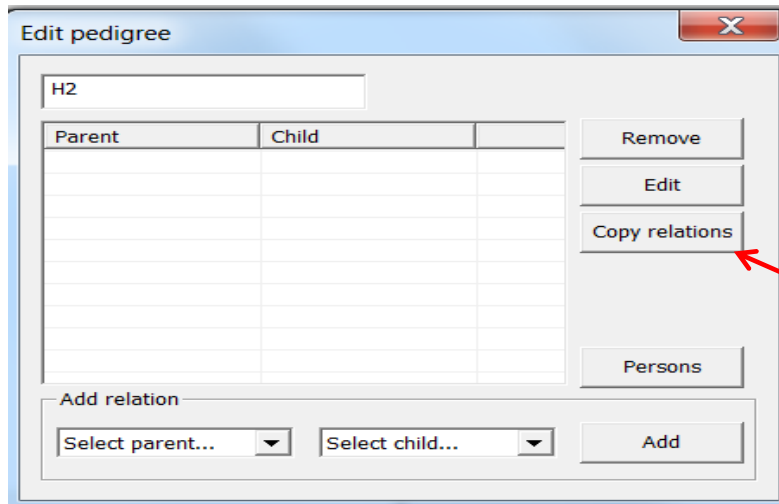
Created pedigree

Creating pedigrees in FamLinkX



Create alternative pedigree

Creating pedigrees in FamLinkX



Copy all relations from first pedigree (H1)

Creating pedigrees in FamLinkX

Edit pedigree

H2

Parent	Child	
Father	Sibling1	
Father	Sibling2	
Father	Sibling3	
Mother	Sibling1	
Mother	Sibling2	
Mother	Sibling3	

Remove
Edit
Copy relations
Persons
Plot in R
Close

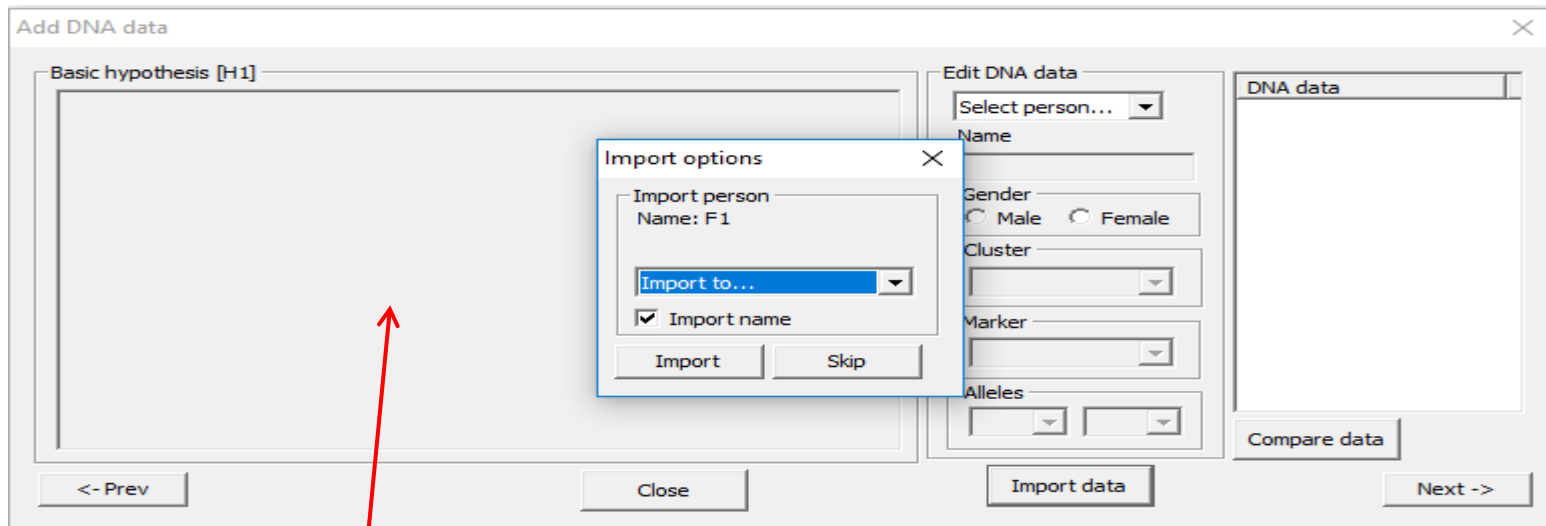
Add relation

Select parent... Select child... Add



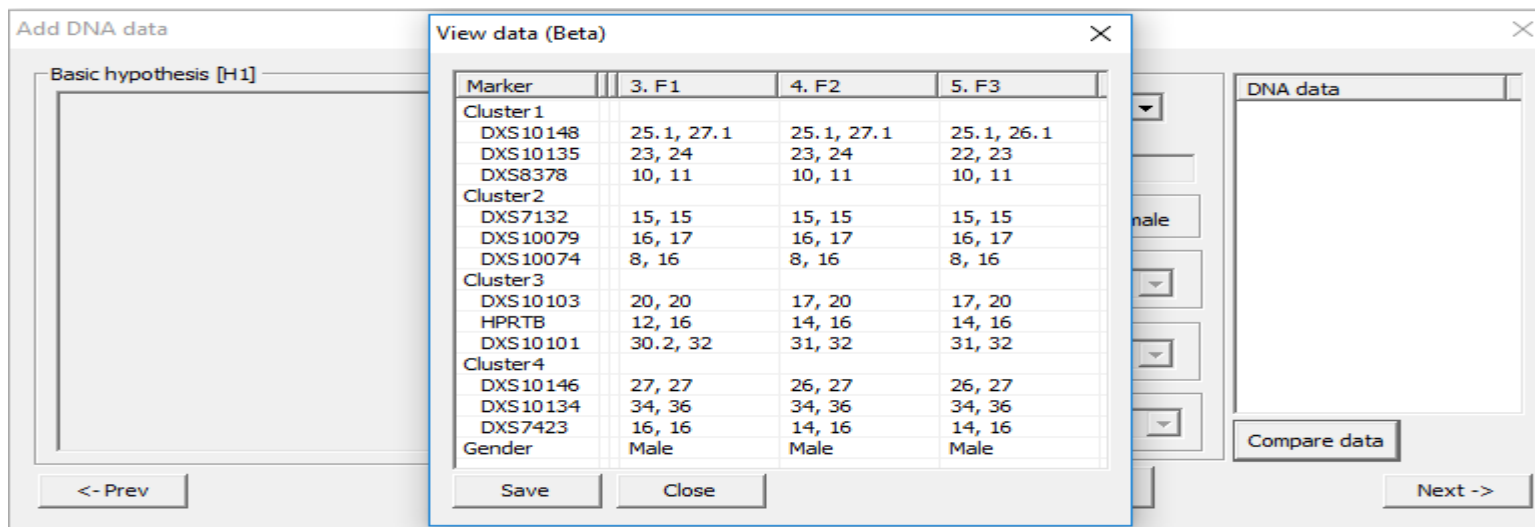
Copy all relations from first pedigree (H1)

Creating pedigrees in FamLinkX



No illustration! (More later)

Creating pedigrees in FamLinkX



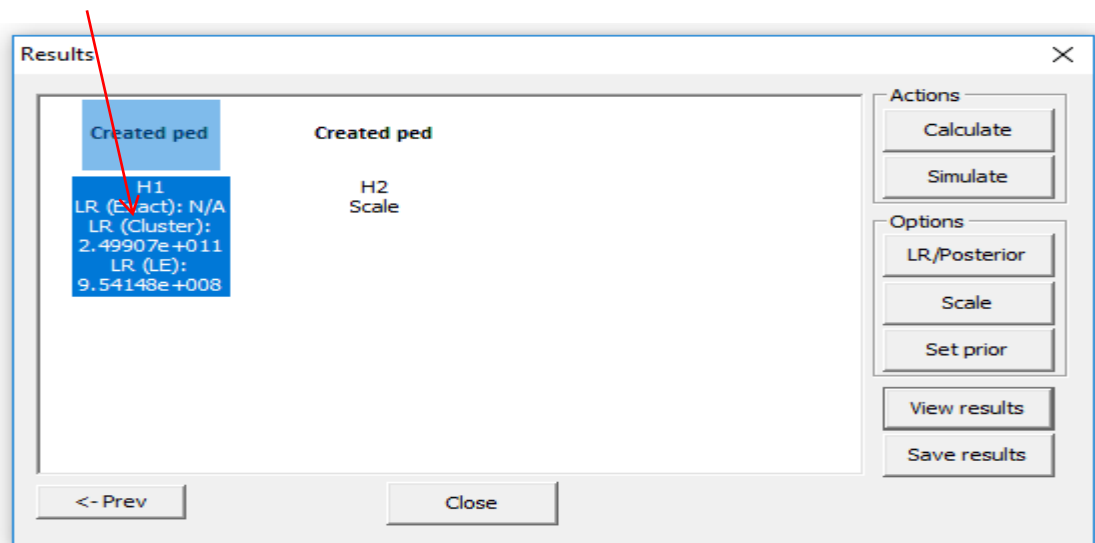
The screenshot displays the 'View data (Beta)' dialog box in the FamLinkX software. The dialog box contains a table with the following data:

Marker	3. F1	4. F2	5. F3
Cluster 1			
DXS10148	25.1, 27.1	25.1, 27.1	25.1, 26.1
DXS10135	23, 24	23, 24	22, 23
DXS8378	10, 11	10, 11	10, 11
Cluster 2			
DXS7132	15, 15	15, 15	15, 15
DXS10079	16, 17	16, 17	16, 17
DXS10074	8, 16	8, 16	8, 16
Cluster 3			
DXS10103	20, 20	17, 20	17, 20
HPRTB	12, 16	14, 16	14, 16
DXS10101	30.2, 32	31, 32	31, 32
Cluster 4			
DXS10146	27, 27	26, 27	26, 27
DXS10134	34, 36	34, 36	34, 36
DXS7423	16, 16	14, 16	14, 16
Gender	Male	Male	Male

The dialog box also features 'Save' and 'Close' buttons at the bottom. The background shows the 'Add DNA data' window with a 'Basic hypothesis [H1]' section and a 'DNA data' section with a 'Compare data' button.

Creating pedigrees in FamLinkX

We should use LR (Cluster)



Creating pedigrees in FamLinkX

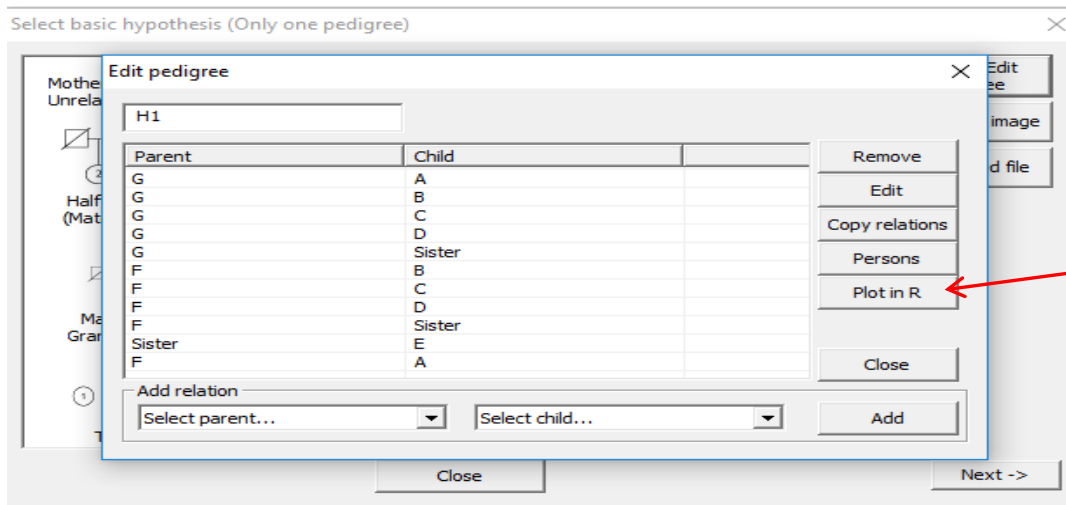
Why does the LR become 0? (or infinity)

- Check pedigree (possible errors)
- Check genders of all persons
- Check for potential mutations
- Advanced users may check the temporary output from Merlin via

C:\Program files (x86)\FamLinkX\temp\tempResults.txt

Plotting in FamLinkX

- Similar as in Familias – requires R
- Available since version 2.8



Plotting in FamLinkX

Select basic hypothesis (Only one pedigree)

Edit pedigree

Parent	Child
G	A
G	B
G	C
G	D
G	Sister
F	B
F	C
F	D
F	Sister
F	E
F	A

Buttons: Remove, Edit, Copy relations, Persons, Plot in R, Close

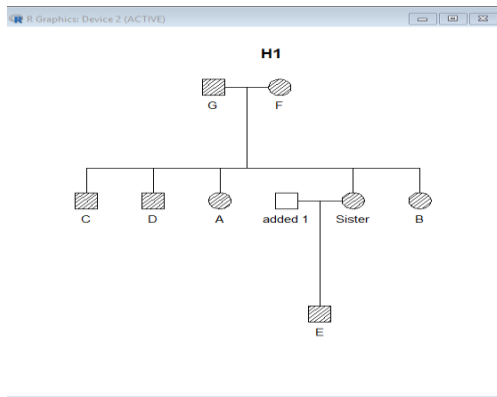
Add relation: Select parent... Select child... Add

Text

The following text may be copied into R to plot the pedigree

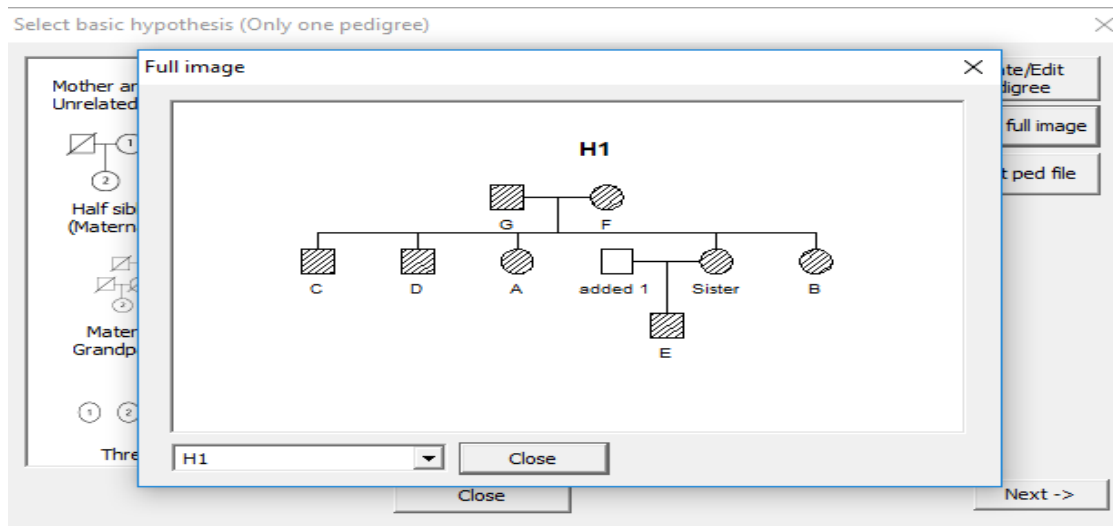
```
# Start the Familias package
foo = library(Familias, logical.return=TRUE)
f(foo)
{
foo2 = FALSE
install.packages("Familias"); foo2 = library(Familias, logical.return=T);
}
#Define the persons involved in the case
persons <- c("G", "F", "C", "D", "E", "A", "Sister", "B")
sex <- c("Female", "Male", "Female", "Female", "Female", "Male", "Male", "Male")
#Define the pedigree
ped1 <- FamiliasPedigree(id=persons, dadid=c(NA,NA,"G","G",NA,"G","G","G"), momid=
pedigrees <- list(ped1)
plot(ped1, affected=c(1,1,1,1,1,1,0), mar = c(4.1, 4, 4.1, 4), cex=0.9, angle=45,
```

Buttons: Save, Close



Plotting in FamLinkX

- Once plotted, it can be viewed in FamLinkX and automatically inserted into report!



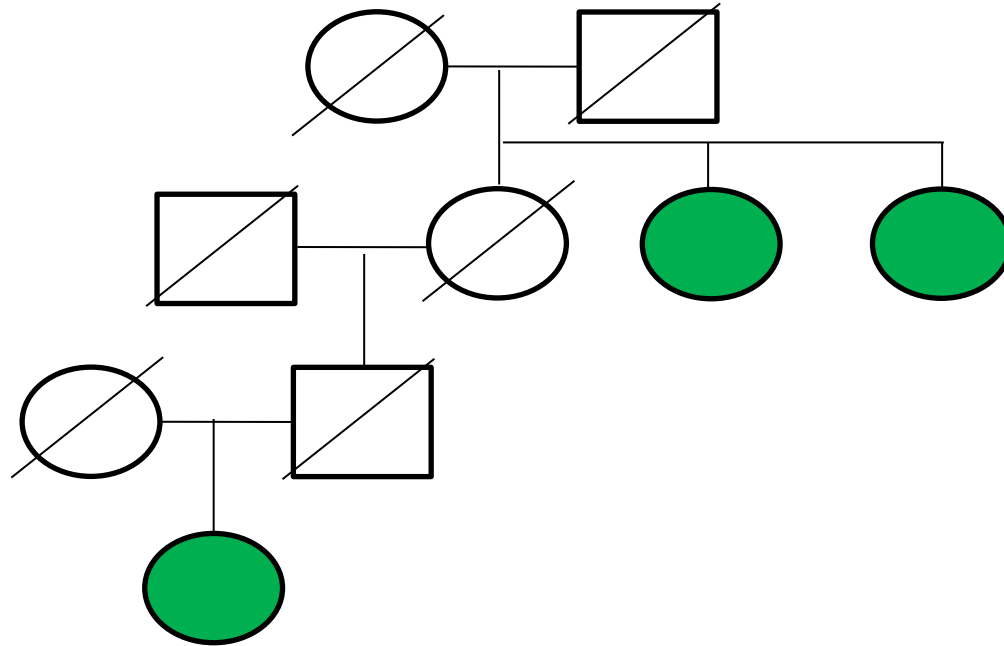


FamLinkX – Creating pedigrees and plotting

DEMONSTRATION 2

Demonstration

➤ Pedigree





UNDERSTANDING THE RESULTS

Understanding the results

➤ FamLinkX computes three different LRs

1. LR (Exact) – Uses the published method in Kling et al.
2. LR (Cluster) – Uses the cluster function in Merlin
3. LR (LE) – Considers the LE model in Merlin (linkage still modelled!)

➤ Why do we present all?

Understanding the results

➤ FamLinkX computes three different LRs

1. LR (Exact) – Uses the published method in Kling et al.
2. LR (Cluster) – Uses the cluster function in Merlin
3. LR (LE) – Considers the LE model in Merlin (linkage still modelled!)

➤ Why do we present all?

- a) Validation purposes
- b) 2 and 3 only available for created peds

Understanding the results

Comparison

	LR (Exact)	LR (Cluster)	LR (LE)	LR (Single marker)
Linkage	Yes	Yes	Yes	No
LD (Haplotypes)	Yes	Yes	No	No
Mutations	Yes	No	No	No
Recombinations within clusters	Yes	No	Yes	NA
Lambda model	Yes	Yes	No	No

Understanding the results

- LR (Cluster) will, in many cases, gives similar results as the LR (Exact)
- LR (LE) will give the same results as the other two when lambda approaches infinity or for Decaplex and similar kits
- If mutations -> Only LR (Exact) will give results
- If recombinations within clusters are necessary -> LR (Exact) and LR (LE) will give results
- If created pedigrees -> Only LR (Cluster) and LR (LE) will give results



ADVANCED SETTINGS

Advanced settings

Lists all pre-defined pedigrees

Pedigree	Threshold	Steps
Duo (Maternity)	0.001	0
Duo (Paternity)	0.001	0
Trio	0.001	0
Unrelated (Duo)	0.001	0
Full Siblings	0.001	0
Half Siblings (Maternal)	0.001	0
Half Siblings (Paternal)	0.001	0
Unrelated	0.001	0
Full Siblings (Data mother)	0.001	0
Half Siblings (Data mothers)	0.001	0
Unrelated (Data mothers)	0.001	0
Grandmother	0.001	0
Grandmother (Data mother)	0.001	0
Aunt/Uncle	0.001	0

Settings

Threshold

Steps (Transitions)

Update

Two parameters

Threshold – Decides what likelihoods should be computed.

Steps – Decides what alleles should be included in the likelihood calculations, ref. stepwise mutation model

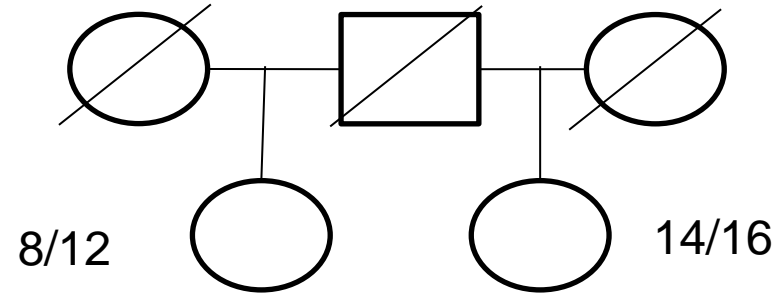
Advanced settings

➤ Example 1

Threshold=0.1, Steps=0

Since steps=0, shared father must have one of the alleles of the daughters. Threshold is high thus discarding low likelihoods, i.e. two step mutations.

LR=0



Threshold=0.000001, Steps=1

Since steps=1, shared father can be 13/13. Threshold allows low likelihoods corresponding to two step mutations as well as double mutations.

LR=0.0035

Advanced settings

➤ Example 2

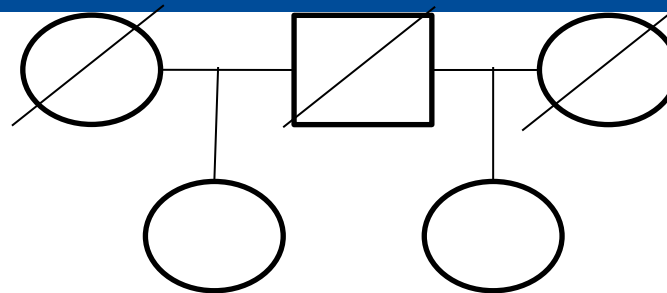
Threshold=0.001, Steps=0

View data (Beta)



Marker	Accumulated LR	Marginal LR	Single mark...
KG1			
DXS 10148	1.96658	1.96658	
DXS 10135	7.95526	4.04521	
DXS8378	25.5163	3.20747	
KG2			
DXS 7132	59.5996	2.33575	
DXS 10079	221.873	3.72273	
DXS 10074	1448.59	6.5289	
KG3			
DXS 10103	3403.55	2.34956	
HPRT	6222.76	1.82832	
DXS 10101	0	0	
KG4			
DXS 10146	0	-1. #IND	
DXS 10134	0	-1. #IND	
DXS 7423	0	-1. #IND	
	0	-1. #IND	1.59541e+...

Save Close



Threshold=0.00001, Steps=0

View data (Beta)



Marker	Accumulated LR	Marginal LR	Single mark...
KG1			
DXS 10148	1.97093	1.97093	
DXS 10135	7.96409	4.04078	
DXS8378	25.5253	3.20505	
KG2			
DXS 7132	59.7143	2.33942	
DXS 10079	222.298	3.7227	
DXS 10074	1451.48	6.52943	
KG3			
DXS 10103	3410.35	2.34956	
HPRT	6244.7	1.8311	
DXS 10101	20.0605	0.0032124	
KG4			
DXS 10146	27.7779	1.38471	
DXS 10134	121.174	4.36225	
DXS 7423	323.422	2.66907	
	323.422	323.422	1.59541e+...

Save Close

Advanced settings

- Keep the steps parameter at zero. Higher values will slow down computations.
- Use a threshold of 0.00001 thus allowing single step mutations.
- Both should be same for all pedigrees and fixed in database. Can be changed if necessary.
- Works on individual markers -> threshold does not have to be extremely low if mutations in different markers.

Other topics (covered later)

- Haplotype frequency model (Lambda)
- Pseudo-counts
- Estimating Lambda from data
- Frequency databases etc.

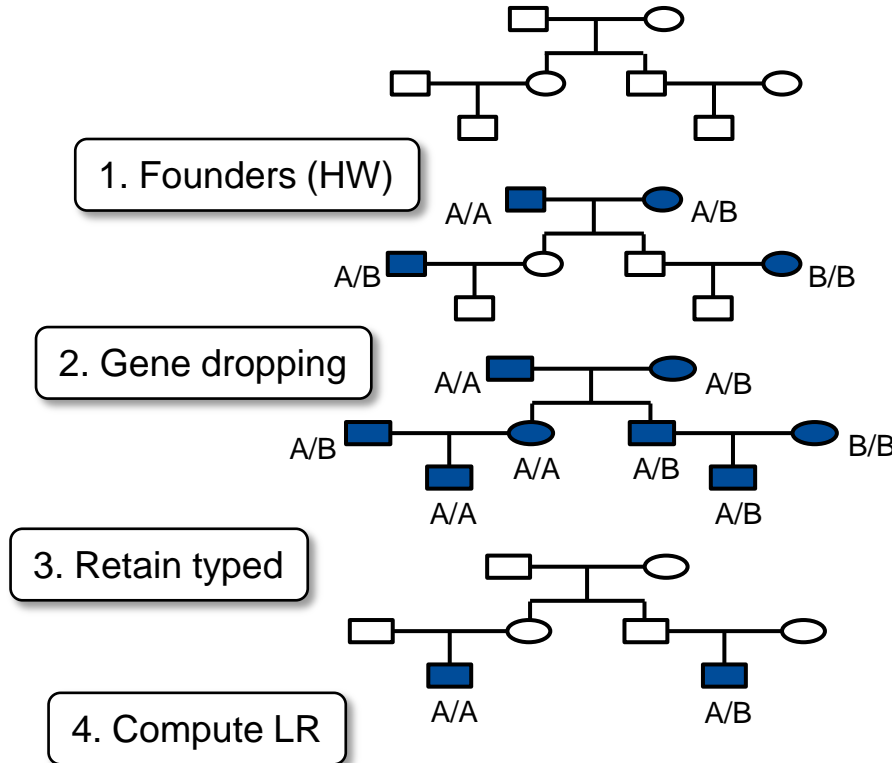


SIMULATIONS

Simulations in FamLinkX

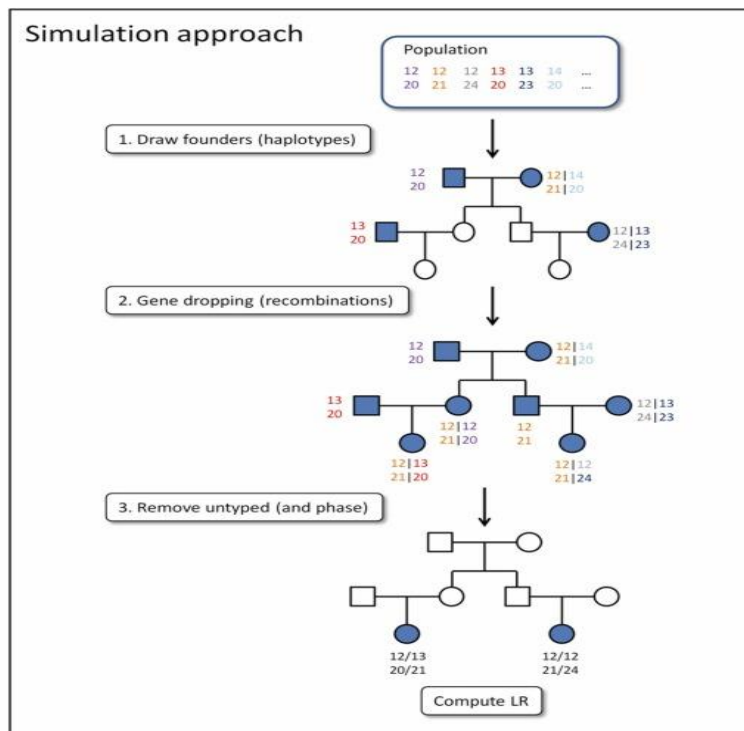
- Similar to Familias
- Gene dropping
- More advanced approach

Simulations – Simple example



A/A	A/B	B/B
0.25	0.5	0.25

Simulations – Full example



Simulations – Output

Simulation	LR (Half sisters)	LR(Unrelated)
1	1000	0.0001
2	100	0.02
3	600	0.1
4	0.1	0.0000001
5	10000	1.5
6	5000	0.01
...

Conclusion?

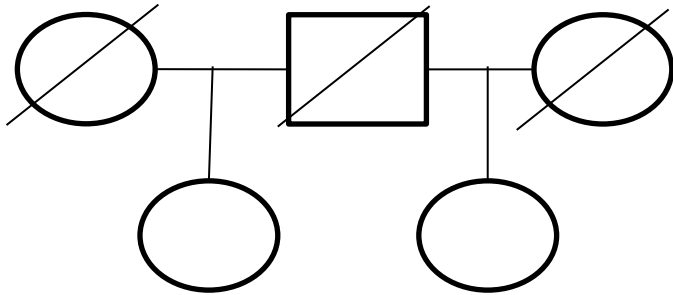


FamLinkX – Simulations

DEMONSTRATION 3

Demonstration

➤ Background

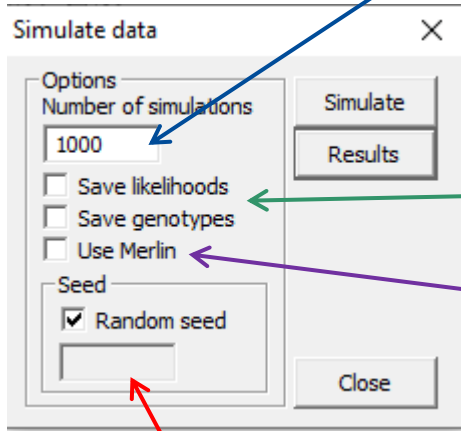


Decaplex data

Simulations in FamLinkX

➤ Settings

#Simulations (at least 100 is recommended)



Save settings

Much faster for Argus X12 (no mutations!)

Set a seed (random)

Simulations in FamLinkX

➤ Results

True pedigree

Summary statistics

Save raw LRs

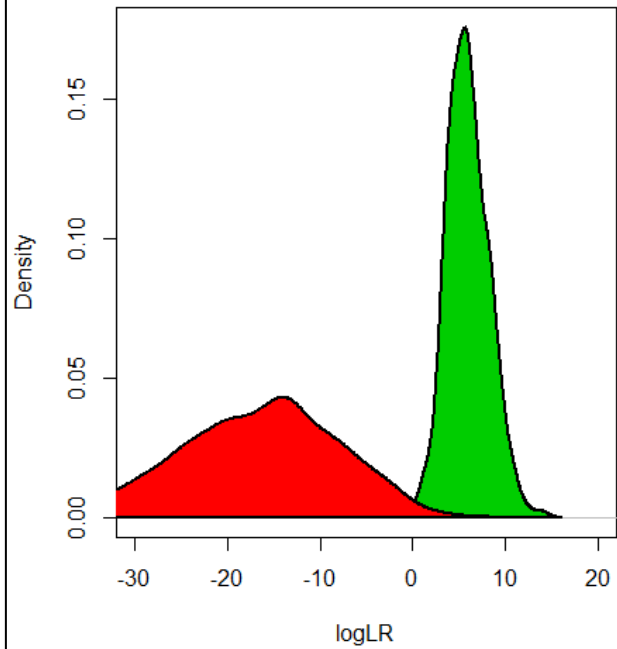
Simulation results - Likelihood ratios

True pedigree	Median	Average	P(LR > 1000)	P(LR > 10000)	P(LR > 100000)
Unrelated	3e-008	0.33	0.000000	0.000000	0.000000
Half Siblings (Pate...)	3.5e+002	9.4e+003	0.319000	0.087000	0.010000

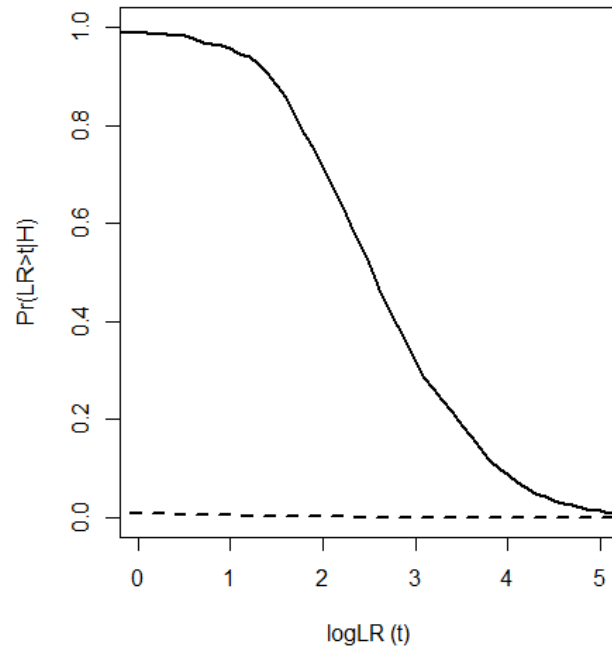
Save LR
Exceedance
Method
1. Exact
Close



A) Distributions

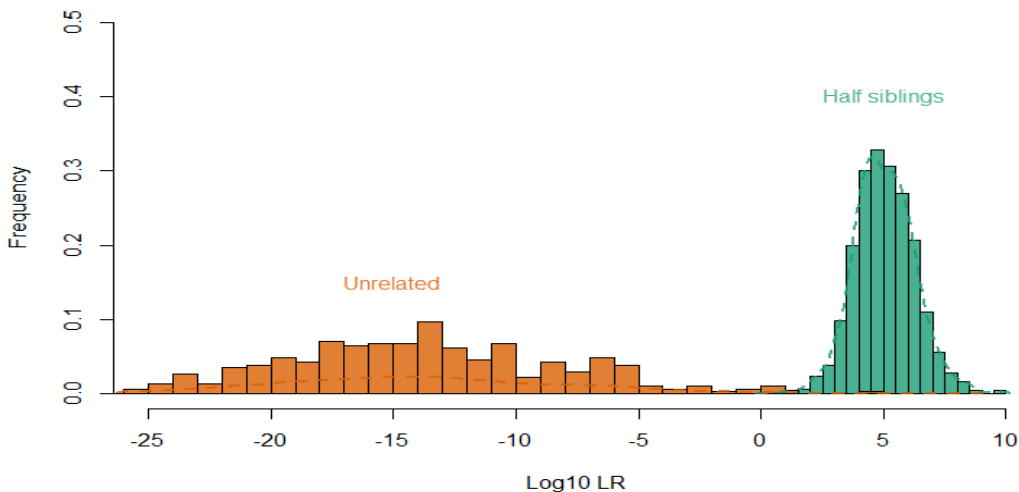


B) Exceedance probabilities



Simulations wth Argus X12

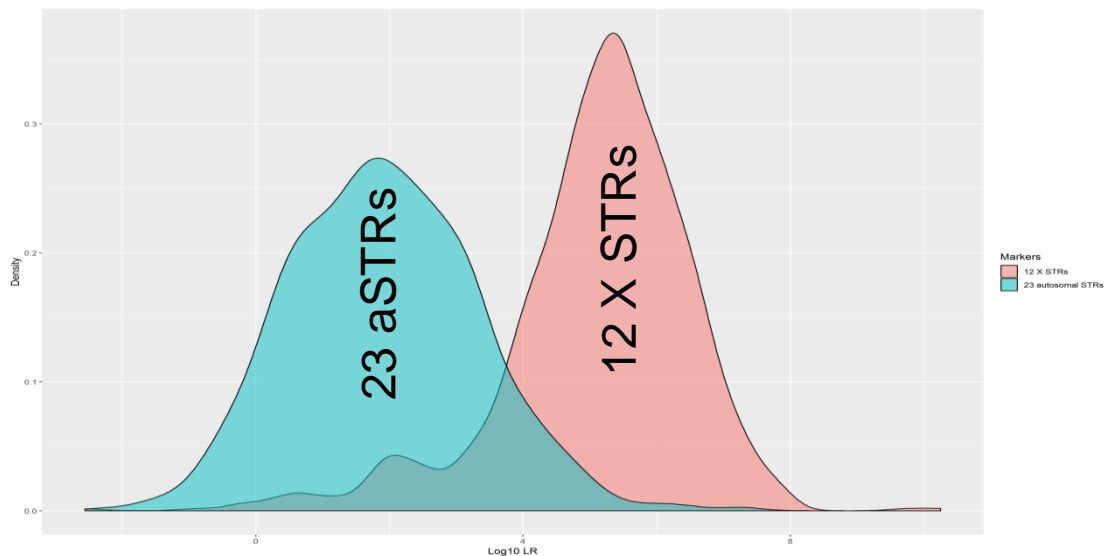
Paternal half sisters (Histogram of 1000 simulations)



Paternal half siblings can be distinguished from unrelated

Autosomal markers vs Argus X12

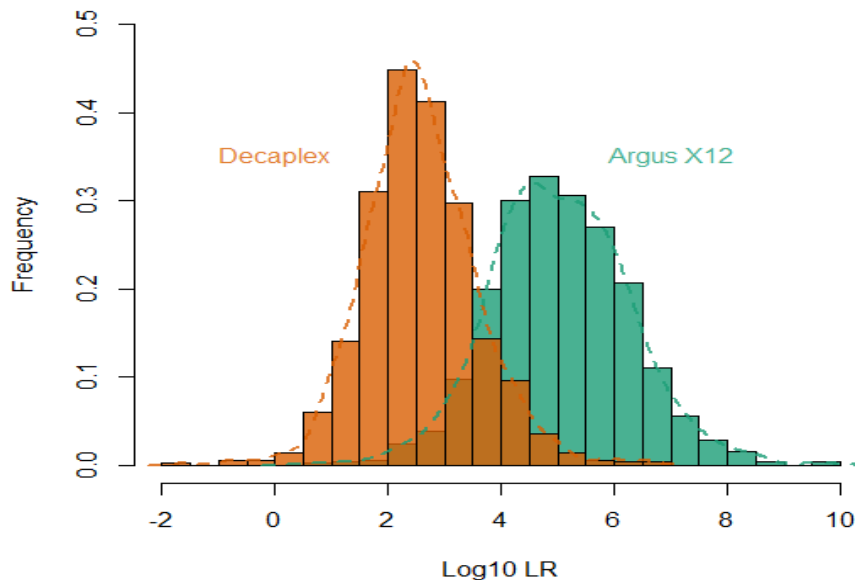
Paternal half sisters



X-STRs is more powerful for some cases

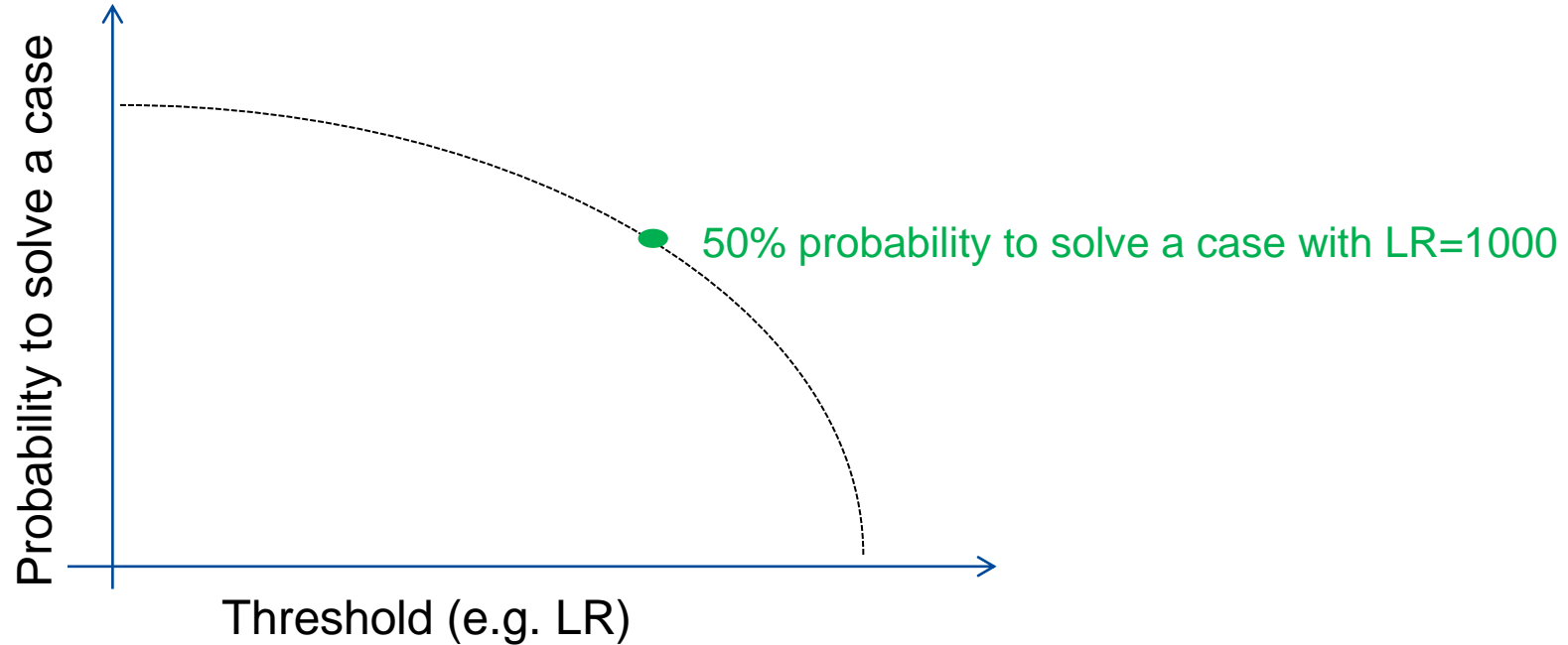
Argus X12 versus Decaplex

Paternal half sisters



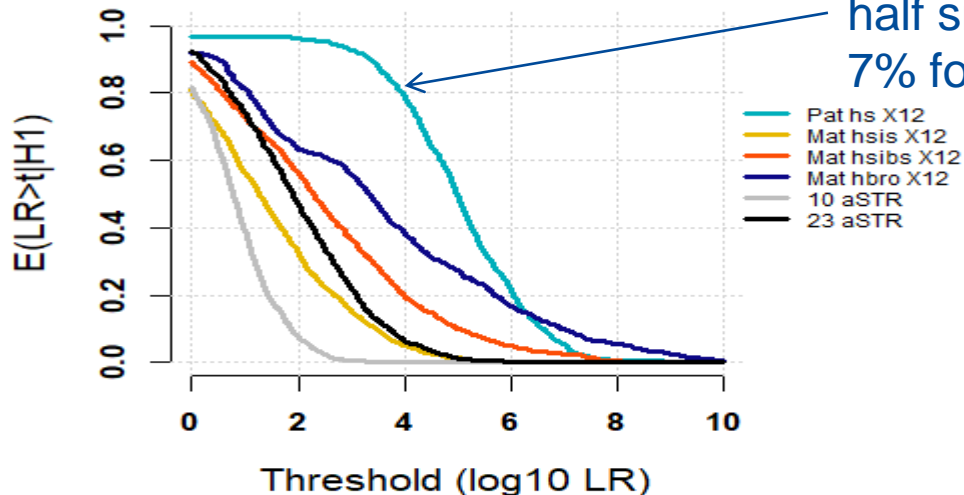
Argus X12 is more powerful than Decaplex

Exceedance plots



Utility of simulations (Argus X12)

B) Half siblings

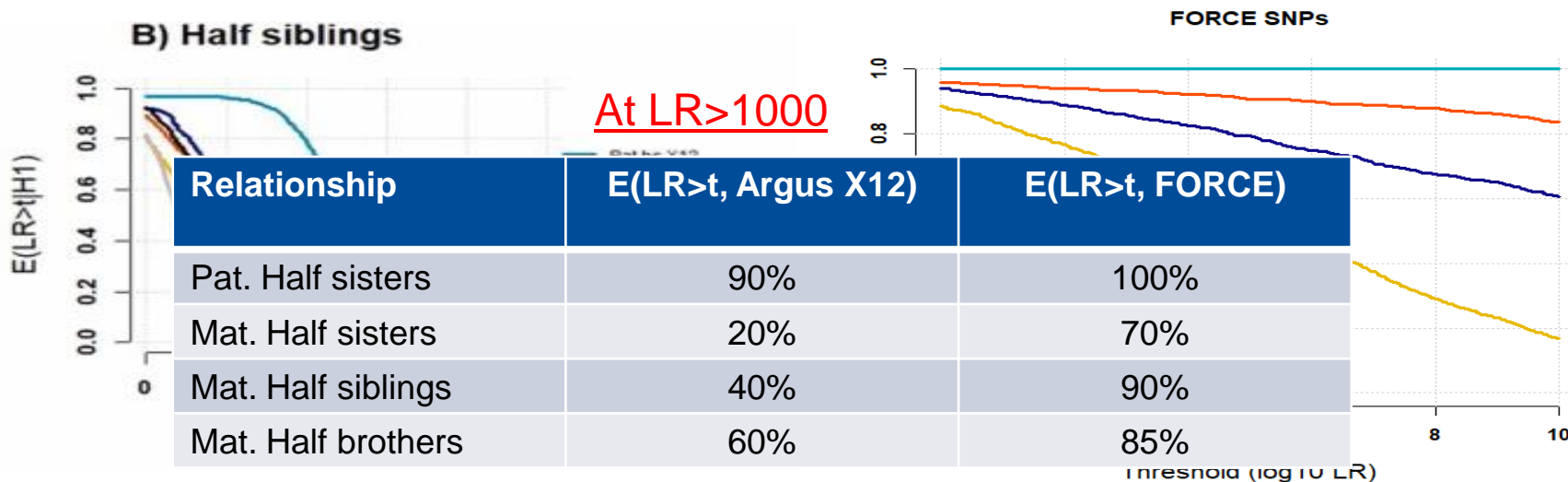


80% chance to solve paternal half siblings with X12 but only 6-7% for 23 aSTR if $LR=10,000$

Probability that we can solve a case with the given threshold

FORCE X-SNPs

242 SNPs (only linkage, no LD)



From Bergseth et al. 2022

Power increased substantially



VALIDATION

Guidelines

The screenshot shows the title page of a research paper. At the top left is the Elsevier logo. The journal title 'Forensic Science International: Genetics' is centered, with the volume and issue information 'Volume 25, November 2016, Pages 191-197' below it. On the top right is the journal's cover image. The paper is identified as a 'Research paper'. The title is 'DNA Commission of the International Society for Forensic Genetics: Recommendations on the validation of software programs performing biostatistical calculations for forensic genetics applications'. The authors are listed as M.D. Coble, J. Buckleton, J.M. Butler, T. Egeland, R. Fimmers, P. Gill, L. Gusmão, B. Guttman, M. Krawczak, N. Morling, W. Parson, N. Pinto, P.M. Schneider, S.T. Sherry, S. Willuweit, and M. Prinz. Below the authors are social media sharing options (Add to Mendeley, Share, Cite) and a DOI link. A 'Highlights' section is visible at the bottom, containing three bullet points.

Forensic Science International: Genetics
Volume 25, November 2016, Pages 191-197

Research paper

DNA Commission of the International Society for Forensic Genetics: Recommendations on the validation of software programs performing biostatistical calculations for forensic genetics applications

M.D. Coble^a, J. Buckleton^{b,c}, J.M. Butler^d, T. Egeland^e, R. Fimmers^f, P. Gill^{g,h}, L. Gusmão^{i,j}, B. Guttman^k, M. Krawczak^m, N. Morlingⁿ, W. Parson^{o,p}, N. Pinto^{j,k,q,r}, P.M. Schneider^s, S.T. Sherry^t, S. Willuweit^u, M. Prinz^v

Show more

+ Add to Mendeley Share Cite

<https://doi.org/10.1016/j.fsigen.2016.09.002> Get rights and content

Highlights

- The International Society for Forensic Genetics (ISFG) has convened a DNA Commission to establish validation guidelines for bio-statistical software to be used in forensic genetics.
- We present recommendations for the minimum requirements to validate bio-statistical software for forensic genetics.
- Recommendations are provided for developmental validation and the comparability of the software developers.

Unfortunately not open access

Guidelines

1. Scientific paper
2. Developmental validation with freely available data
3. Version control
4. Instruction for how to validate (internal validations)
5. User manual
6. Training and education
7. Open source/algorithms
8. Updates/bug fixes etc

Guidelines

9. Randomness (MCMC etc)
10. Plan for internal validation – which tests to perform etc
11. Test the software on in-house data from real samples
12. Samples to include
13. Consistent with previous results (e.g. other software)
14. Standard operating procedure (SOP)
15. In-house training
16. Public repository

Guidelines

The screenshot shows the abstract page of a paper titled "FamLinkX – implementation of a general model for likelihood computations for X-chromosomal marker data" published in *Forensic Science International: Genetics*, Volume 17, July 2015, Pages 1-7. The authors listed are Daniel Kling, Barbara Dell'Amico, and Andreas O. Tillmar. The abstract highlights that a general implementation for likelihood calculations on X-chromosomal marker data is presented, that the model includes linkage disequilibrium and mutations, that the software is freely available at www.famlink.se, and that validation and theoretical derivations are provided.

Forensic Science International: Genetics
Volume 17, July 2015, Pages 1-7

FamLinkX – implementation of a general model for likelihood computations for X-chromosomal marker data

Daniel Kling ^{a b} ✉, Barbara Dell'Amico ^c ✉, Andreas O. Tillmar ^{c d} ✉

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<https://doi.org/10.1016/j.fsigen.2015.02.007> [Get rights and content](#)

Highlights

- A general implementation for likelihood calculations on X-chromosomal marker data is presented.
- We model linkage, [linkage disequilibrium](#) as well as mutations.
- The implementation is freely available at www.famlink.se.
- Concordance with other software, where applicable, is demonstrated.
- Validation and theoretical derivations for some calculations are provided.

Unfortunately not open access. Ask us for a copy!

Guidelines for FamLinkX

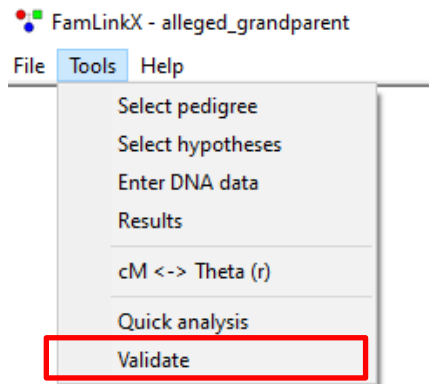
1. Import of data
 - a) Importing frequencies
 - b) Importing haplotypes
 - c) Importing case-specific DNA data (new alleles)
2. Test parameters (value of lambda)
3. Test all relevant cases (hypotheses/pedigrees)
4. Report (output)
5. Save/Open projects with same results
6. Identify sources/points where errors can arise

Guidelines for FamLinkX

7. Create pedigrees
8. Training and education

Simulation does not generally need to be validated

Self-validation in FamLinkX



Will test the pre-defined pedigrees through

1. Two genetic markers (no mutations)
2. 100 simulations
3. Compare results with Merlin



FamLinkX

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GHEP-OS Spring 2025

● WORKSHOP 1

X-chromosomal markers in forensic genetics
Daniel Kling & Andreas Tillmar

● WORKSHOP 2

Acreditación en el campo de la Genética Forense y estrategias de validación de ensayos
Manuel Crespillo Márquez, Rosalía Izquierdo & Estel Enreig Cabanes

● WORKSHOP 3

La genética en la Identificación de víctimas a gran escala: comparación de perfiles y evaluación estadística con Familias
Carlos Vullo & Lourdes Prieto