

Kinship testing with linked markers

Magnus D. Vigeland

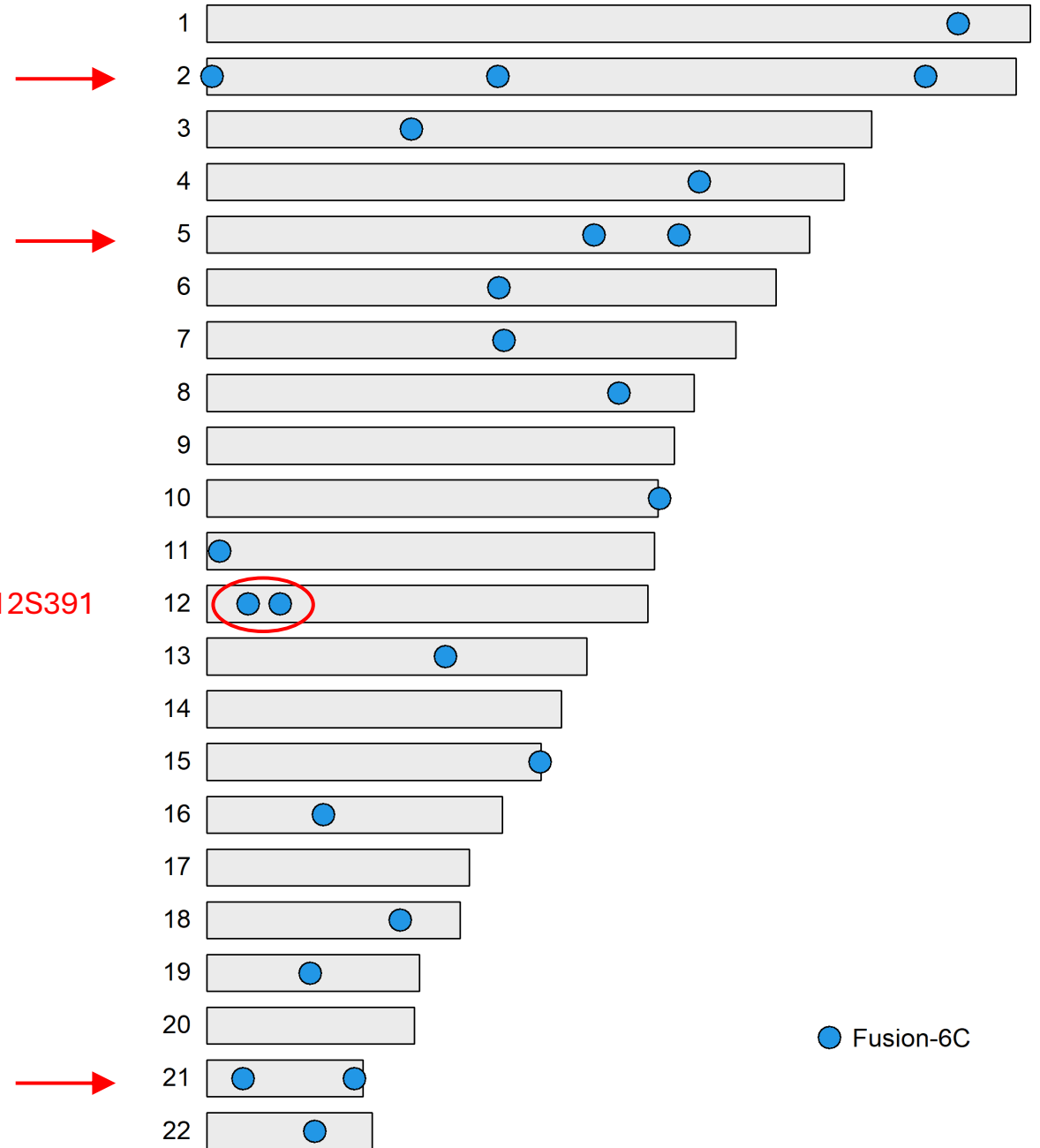
Dept. of Forensic Sciences, Oslo University Hospital

Workshop - Alcalá de Henares - 2026

PowerPlex® Fusion 6C

- 23 autosomal STR markers
- 1 close pair (< 25 cM)
- 4 linkage groups

vWA – D12S391

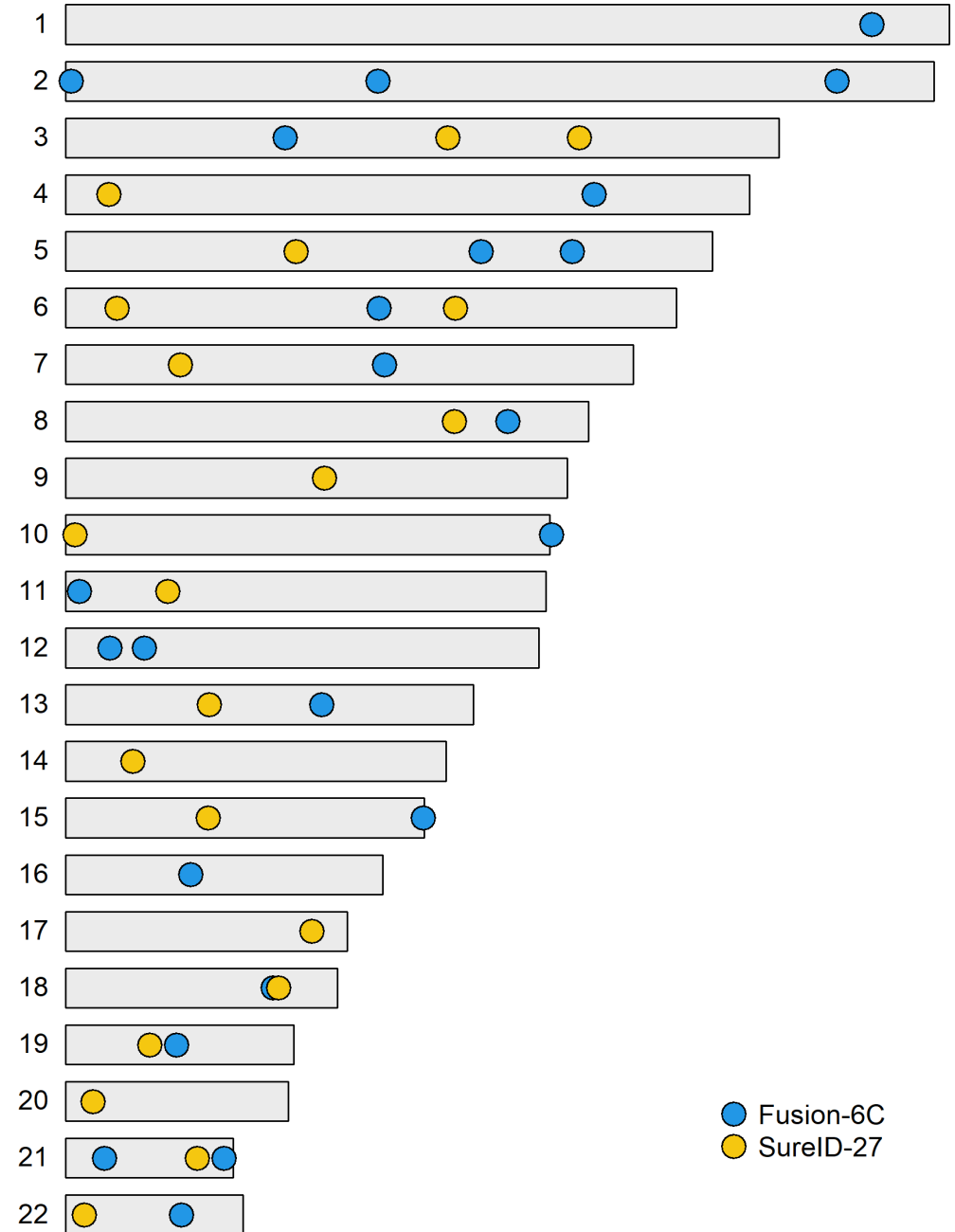


PowerPlex® Fusion 6C

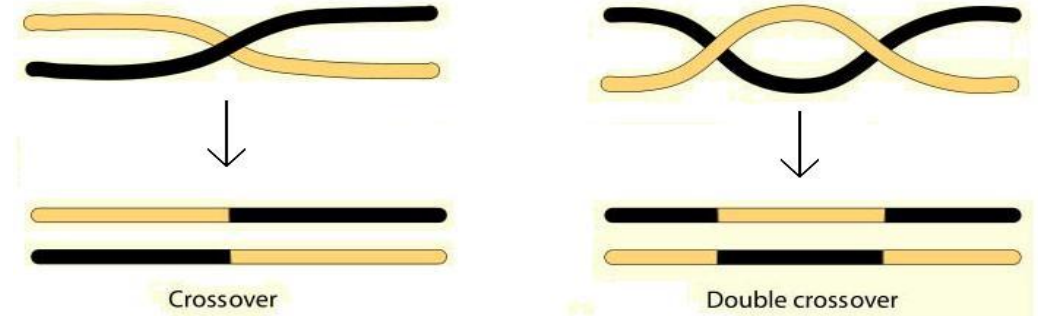
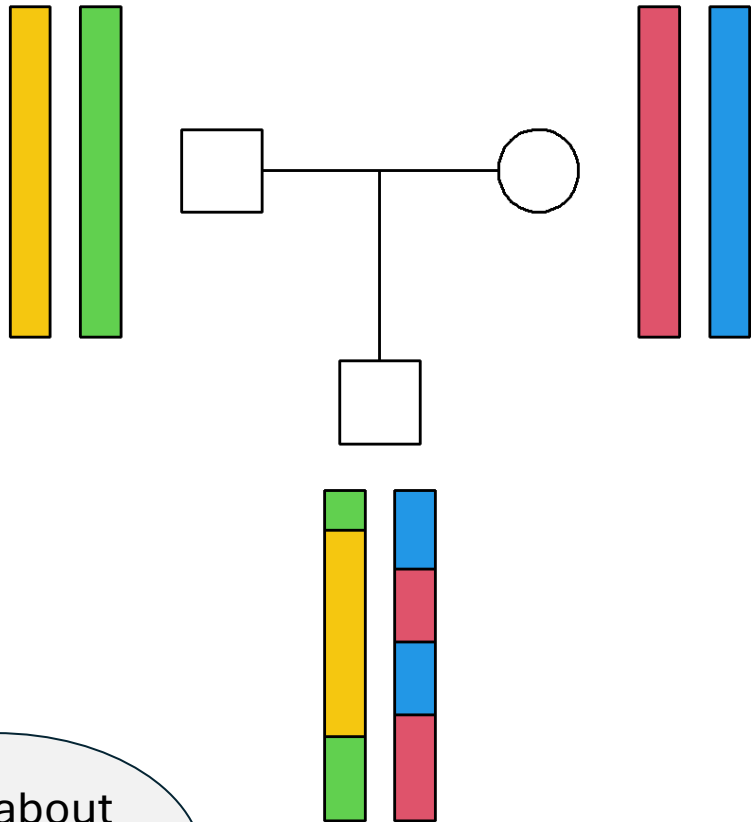
- 23 autosomal STR markers
- 1 close pair (< 25 cM)
- 4 linkage groups

Fusion 6C + SureID® 27

- 43 markers
- 6 close pairs (< 25 cM)
- 16 linkage groups



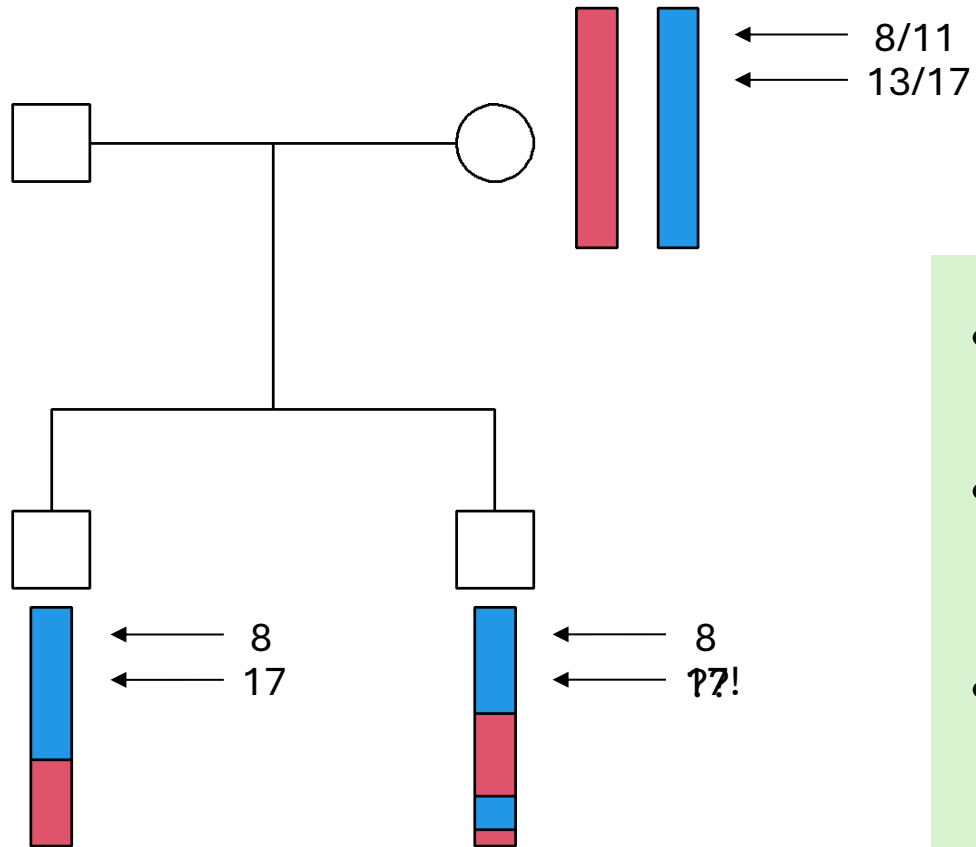
Meiotic recombination



- **Genetic distance** between two loci:
= average crossovers per meiosis
- Units:
 - 1 Morgan (M) = 1 crossover per meiosis
 - 1 centiMorgan (cM) = 0.01 M
- The human genome: Ca 30 Morgan

More about
this
tomorrow!

Why do we care about linkage?



- Linkage \rightarrow dependent inheritance
- Irrelevant in paternity cases (duo/trio)
(unless phased data)
- Relevant if ≥ 2 informative,
non-independent meioses

Software handling linked markers

(No linkage)



MERLIN

Pros

- **Gold standard** for linkage
- Arbitrary linkage

Cons

- Command-line based
- Max 30 alleles
- No mutation modelling

FamLink2

Pros

- Designed for forensics
- Good with SNPs
- GUI + many features
- *Quick analysis* mode

Cons

- No mut. models in QA

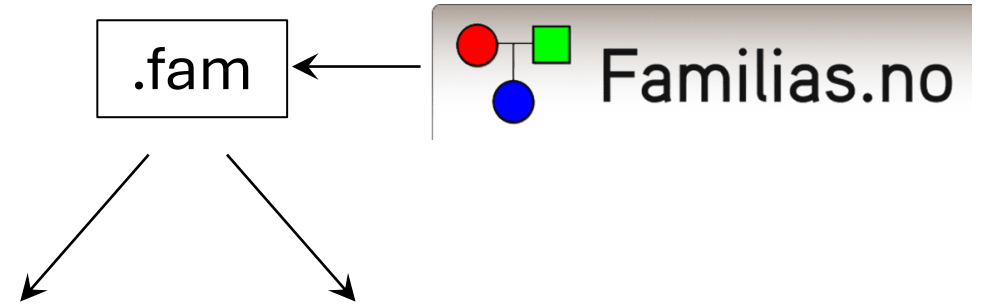
KLINK

Pros

- Designed for kinship STRs
- GUI with visualisations
- Any mutation models
- Tailor-made Excel reports

Cons

- Only pairwise linkage





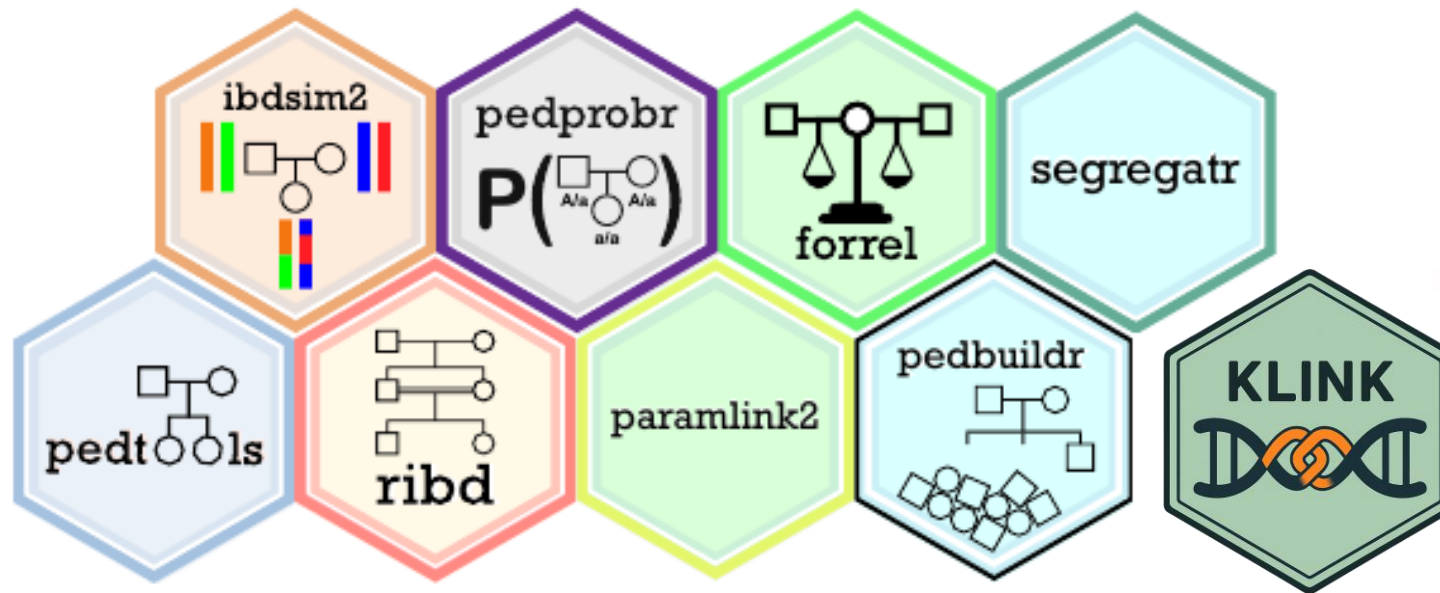
Home page: <https://magnusdv.github.io/pedsuite>

Source code: <https://github.com/magnusdv>



KLINK part of the **pedsuite**:

A collection of packages for pedigree analysis in R



Siv
Gilfillan

KLINK is available in two ways:

- From R: `KLINK::launchApp()`
- Online: <https://magnusdv.shinyapps.io/klink/>

PEDIGREE ANALYSIS IN R



Magnus Dehli Vigeland





INPUT

RESET

Load .fam file

Upload complete

Marker map

Built-in Custom

(Optional) .xml

Example 1

Example 2

SETTINGS

Map function

Kosambi Haldane

Empty markers

Hide Show

Likelihoods

Hide Show Loglik

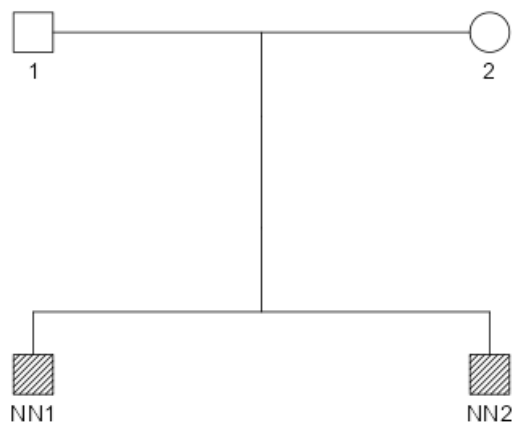
Decimals

Ignore linkage above (cM)

Calculate LR

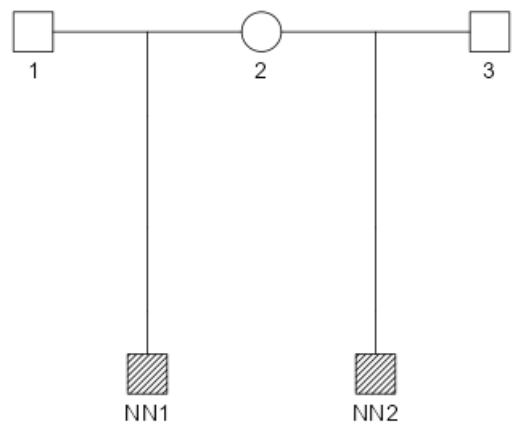
Ped 1

Hide untyped components



Ped 2

Marker



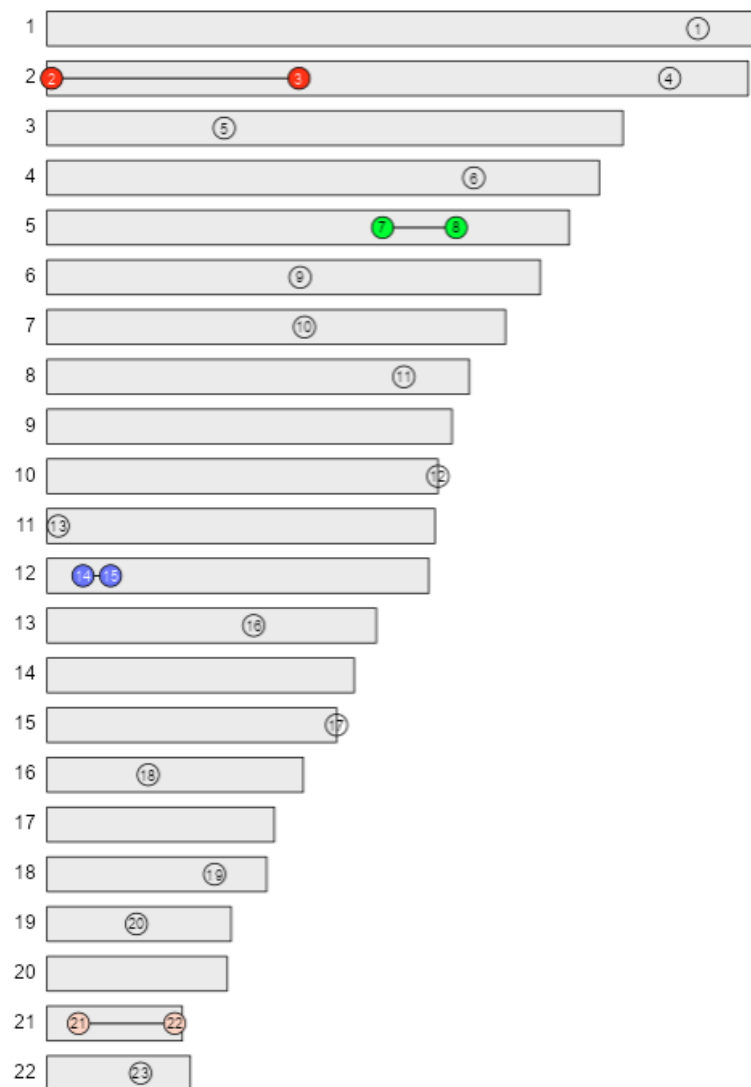
Linkage map

Marker data

LR table

Download

Marker	Chr	cM	Kit
1 D1S1656	1	244.235	Fusion6C/SureID
2 TPOX	2	1.666	Fusion6C
3 D2S441	2	90.479	Fusion6C/SureID
4 D2S1338	2	223.483	Fusion6C
5 D3S1358	3	67.179	Fusion6C
6 FGA	4	156.813	Fusion6C
7 D5S818	5	126.673	Fusion6C
8 CSF1PO	5	154.434	Fusion6C
9 SE33	6	95.449	Fusion6C
10 D7S820	7	100.201	Fusion6C
11 D8S1179	8	136.443	Fusion6C
12 D10S1248	10	169.899	Fusion6C/SureID
13 TH01	11	4.489	Fusion6C
14 vWA	12	15.630	Fusion6C
15 D12S391	12	27.571	Fusion6C/SureID
16 D13S317	13	79.831	Fusion6C
17 Penta E	15	124.051	Fusion6C
18 D16S539	16	50.000	Fusion6C/SureID
19 D18S51	18	88.921	Fusion6C
20 D19S433	19	51.726	Fusion6C
21 D21S11	21	14.646	Fusion6C
22 Penta D	21	59.376	Fusion6C
23 D22S1045	22	46.214	Fusion6C/SureID





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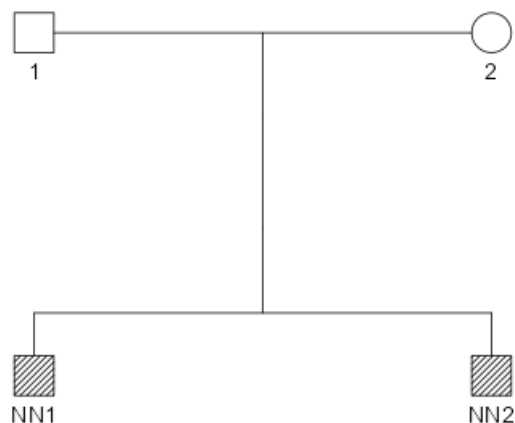
Decimals

Ignore linkage above (cM)

Calculate LR

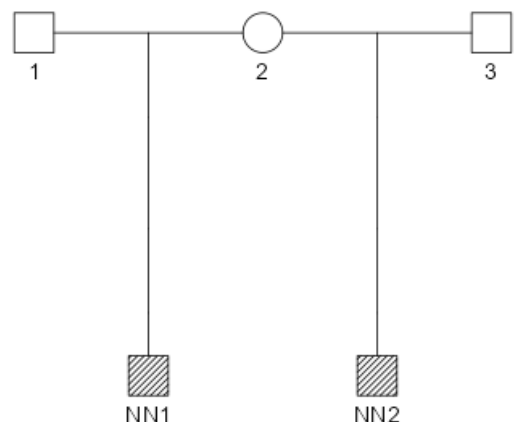
Ped 1

Hide untyped components



Ped 2

Marker



Linkage map

Marker data

LR table

Download

Marker	Person1	Person2	Alleles	PIC	MinFreq	Model	Rate	Rate2	Range	Stat	Lump
D1S1656	15.3/16	15/15.3	17	0.891	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
TPOX	8/9	8/8	9	0.569	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D2S441	10/14	10/14	13	0.696	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D2S1338	17/20	24/24	13	0.863	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D3S1358	14/18	14/18	12	0.766	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
FGA	20/21	20/21	25	0.855	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D5S818	9/12	9/12	9	0.645	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
CSF1PO	13/13	13/13	11	0.688	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
SE33	25.2/28.2	20/21	55	0.947	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D7S820	8/12	8/12	19	0.794	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D8S1179	14/14	14/14	12	0.779	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D10S1248	13/14	13/16	9	0.718	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
TH01	6/9.3	6/9.3	10	0.734	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
vWA	14/20	14/20	12	0.775	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D12S391	18/19.3	19.3/21	23	0.884	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D13S317	11/12	12/12	9	0.779	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
Penta E	7/11	11/16	21	0.890	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D16S539	11/11	11/11	9	0.737	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D18S51	12/15	12/15	23	0.866	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D19S433	12/13	12/13	17	0.739	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D21S11	27/28	27/31	26	0.822	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
Penta D	10/12	10/12	24	0.807	0.001	Stepwise	0.001/0.002	1e-06	0.1	No	No
D22S1045	11/15	15/16	9	0.674	0.0012	Stepwise	0.001/0.002	1e-06	0.1	No	No



INPUT

RESET

Load .fam file

Upload complete

Marker map

Built-in Custom

(Optional) .xml

Example 1

Example 2

SETTINGS

Map function

Kosambi Haldane

Empty markers

Hide Show

Likelihoods

Hide Show Loglik

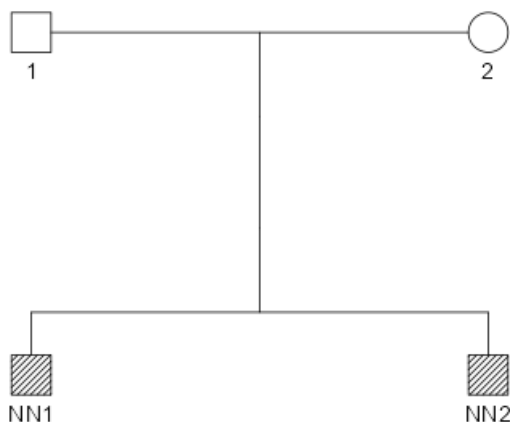
Decimals

Ignore linkage above (cM)

Calculate LR

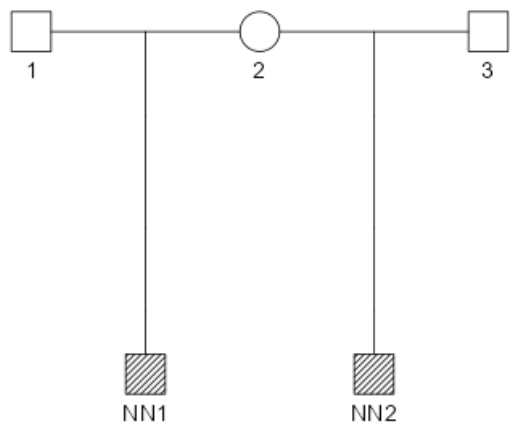
Ped 1

Hide untyped components



Ped 2

Marker



Linkage map

Marker data

LR table

Download

Marker	Person1	Person2	LR	Linked	No link	No mut
D1S1656	15.3/16	15/15.3	0.882	0.882	0.882	0.874
TPOX	8/9	8/8	0.742	1.720	1.722	1.713
D2S441	10/14	10/14	2.322			
D2S1338	17/20	24/24	0.500	0.500	0.500	0.500
D3S1358	14/18	14/18	3.787	3.787	3.787	3.799
FGA	20/21	20/21	3.192	3.192	3.192	3.202
D5S818	9/12	9/12	3.205	23.903	21.215	24.158
CSF1PO	13/13	13/13	6.618			
SE33	25.2/28.2	20/21	0.500	0.500	0.500	0.500
D7S820	8/12	8/12	3.448	3.448	3.448	3.457
D8S1179	14/14	14/14	2.813	2.813	2.813	2.822
D10S1248	13/14	13/16	0.721	0.721	0.721	0.721
TH01	6/9.3	6/9.3	2.011	2.011	2.011	2.012
vWA	14/20	14/20	9.499	4.531	9.353	4.532
D12S391	18/19.3	19.3/21	0.985			
D13S317	11/12	12/12	0.819	0.819	0.819	0.816
Penta E	7/11	11/16	0.857	0.857	0.857	0.858
D16S539	11/11	11/11	2.129	2.129	2.129	2.133
D18S51	12/15	12/15	3.724	3.724	3.724	3.735
D19S433	12/13	12/13	3.705	3.705	3.705	3.722
D21S11	27/28	27/31	0.932	2.674	2.780	2.680
Penta D	10/12	10/12	2.984			
D22S1045	11/15	15/16	0.714	0.714	0.714	0.714
Total LR				2.75×10^5	5.25×10^5	2.80×10^5



INPUT

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Upload complete

Marker map

Built-in Custom

(Optional) .xml

Example 1

Example 2

SETTINGS

Map function

Kosambi Haldane

Empty markers

Hide Show

Likelihoods

Hide Show Loglik

Decimals

Ignore linkage above (cM)

Calculate LR

Ped 1



Ped 2



KLINK-sibship.xlsx

File Home Insert Page Layout Formulas Data Review View Automate Help Acrobat

A1 :

	A	B	C	D	E	F	G	H	I
1	Resultatrapport: sibship								
2		Markør	Person1	Person2	LR				Samples
3	1	D1S1656	15.3-16	15-15.3	0.882				Person1 NN1
4	2	D2S1338	17-20	24-24	0.500				Person2 NN2
5	3	D3S1358	14-18	14-18	3.787				
6	4	FGA	20-21	20-21	3.192				
7	5	SE33	25.2-28.2	20-21	0.500				Relationship
8	6	D7S820	8-12	8-12	3.448				Ped 1 Full siblings
9	7	D8S1179	14-14	14-14	2.813				Ped 2 Half siblings
10	8	D10S1248	13-14	13-16	0.721				
11	9	TH01	6-9.3	6-9.3	2.011				
12	10	D13S317	11-12	12-12	0.819				Notifications
13	11	Penta E	7-11	11-16	0.857				No notifications recorded
14	12	D16S539	11-11	11-11	2.129				
15	13	D18S51	12-15	12-15	3.724				Settings
16	14	D19S433	12-13	12-13	3.705				1 KLINK version: 1.1.0
17	15	D22S1045	11-15	15-16	0.714				2 Familias file: sibship.fam
18	16	TPOX	8-9	8-8	1.720	0.742			3 Genetic map: BUILT-IN
19	17	D2S441	10-14	10-14		2.322			4 Database: NorskDB_2023
20	18	D5S818	9-12	9-12	23.903	3.205			5 Map function: Kosambi
21	19	CSF1PO	13-13	13-13		6.618			6 Max distance: 1000 cM
22	20	vWA	14-20	14-20	4.531	9.499			
23	21	D12S391	18-19.3	19.3-21		0.985			
24	22	D21S11	27-28	27-31	2.674	0.932			
25	23	Penta D	10-12	10-12		2.984			
26	Total LR				2.752e+05				
27									
28									
29									

Linkage map Marker data LR table Notifications Full report Plots Unlinked report

No mut

0.874
1.713

0.500
3.799
3.202
24.158

0.500
3.457
2.822

0.721
2.012
4.532

0.816
0.858

2.133
3.735
3.722
2.680

0.714
2.80 × 10⁵

Download

KLINK: Kinship with pairwise linked markers

Most options can be left untouched

Click on question marks to see explanations

Mutation modelling:

- "Original" = use models in the .fam file (if any)
- "Simple" = Equal model with rate 0.001 (all markers)

INPUT RESET ?

Families .fam file
No file selected

Optional .xml file
No file selected

Example 1 Example 2

SETTINGS ?

Marker map
 Built-in Custom

Map function
 Kosambi Haldane

Mutation model
 Original Simple Off

Empty markers
 Hide Show

Likelihoods
 Hide Show Loglik

Decimals **Unlinked > cM**

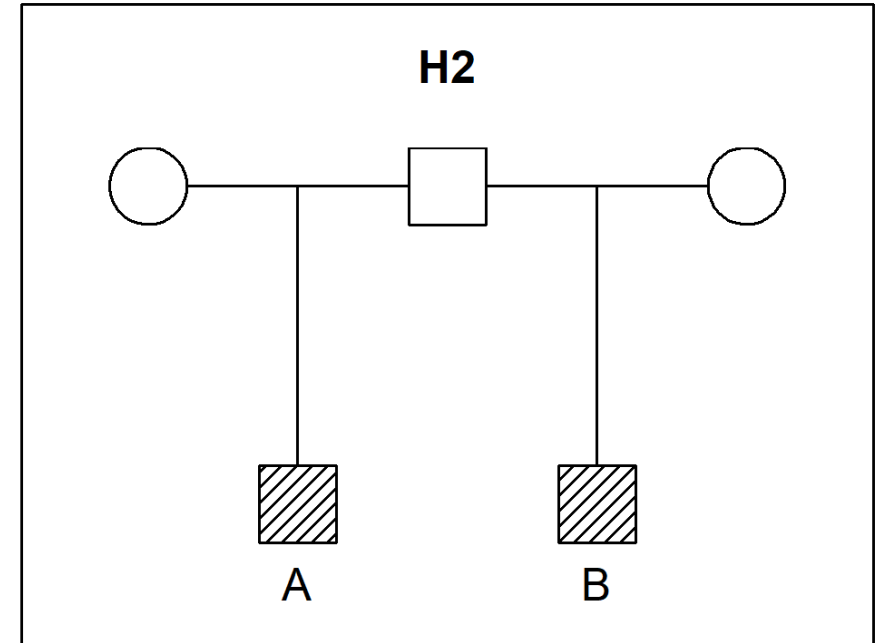
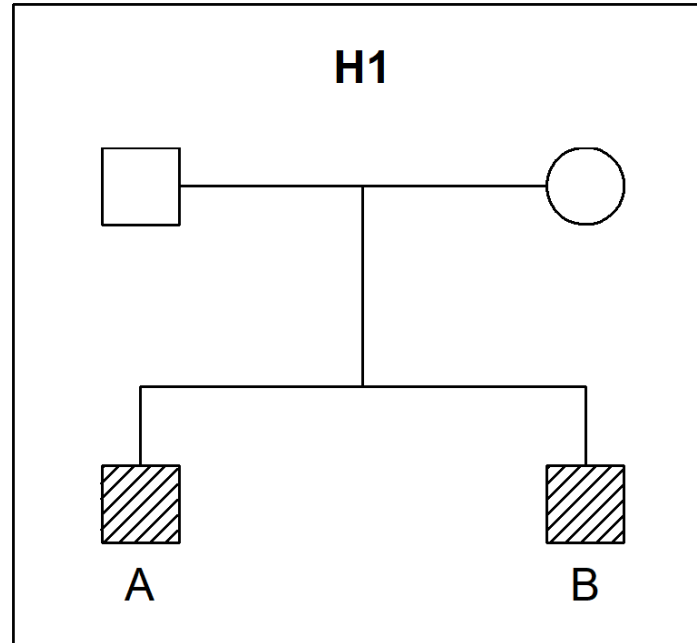
Ped 1

Ped 2

But how much does linkage matter?



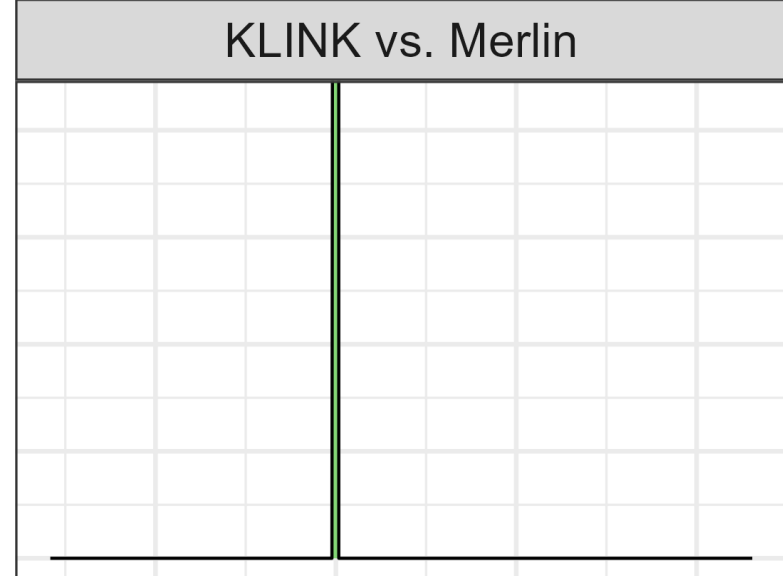
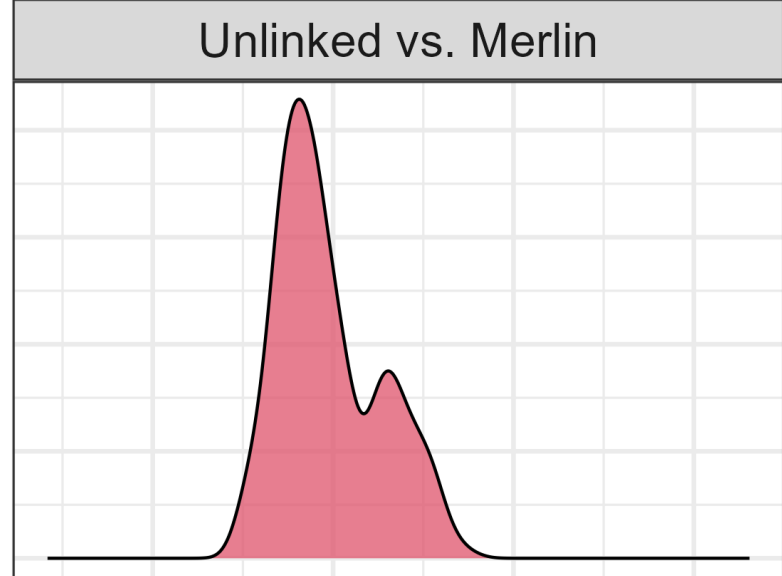
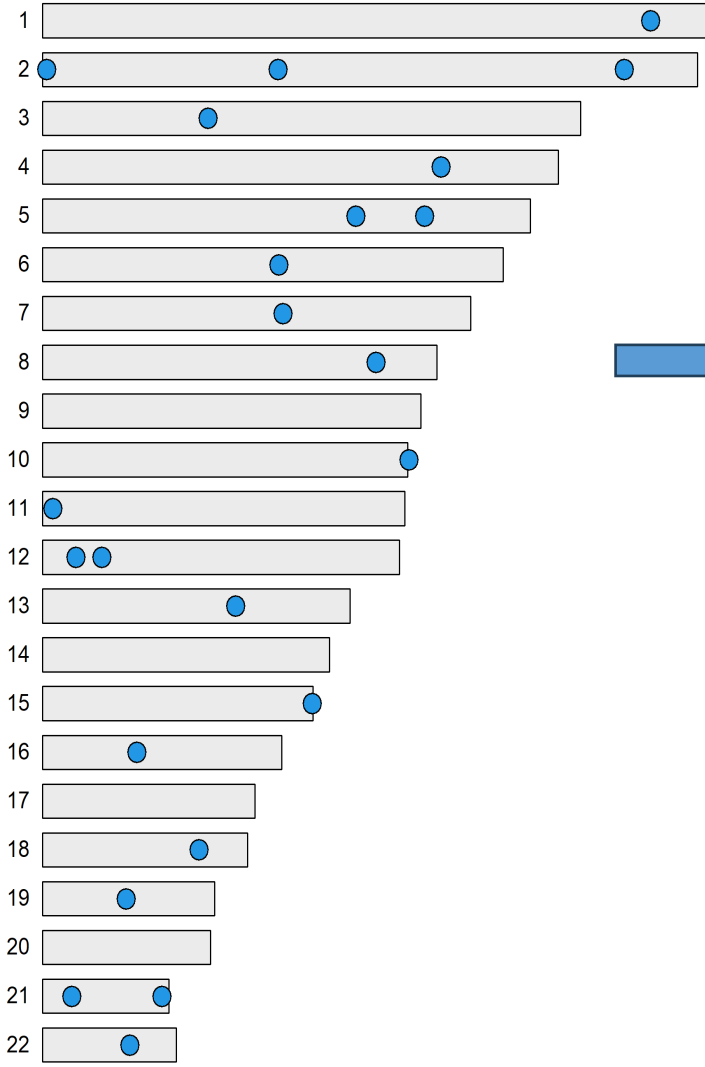
Example 1



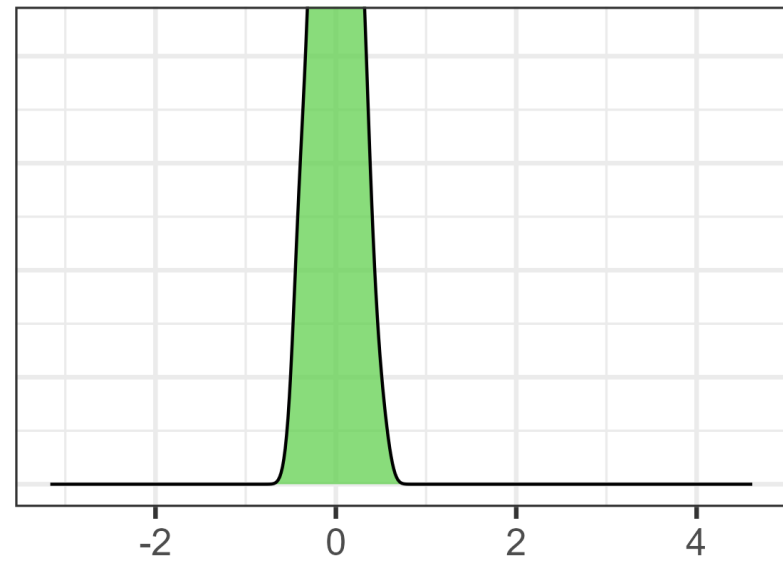
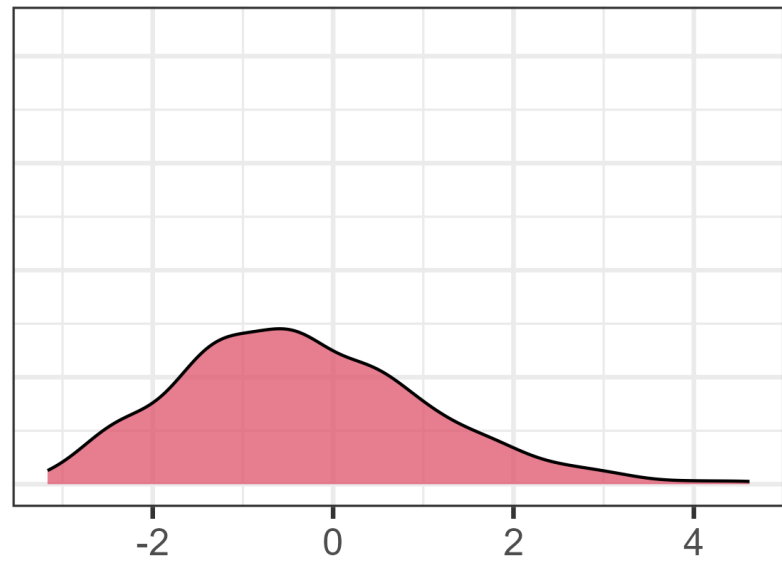
Simulation experiment

- 500 profiles for A and B under H1
- Sims respect linkage
- Marker sets
 - Fusion6c (23 markers)
 - Fusion6c + SureID (43 markers)
- Compute LR with
 - No linkage
 - Pairwise linkage (KLINK)
 - Full linkage (Merlin = FamLink2)

All analyses done in R with the **pedsuite**

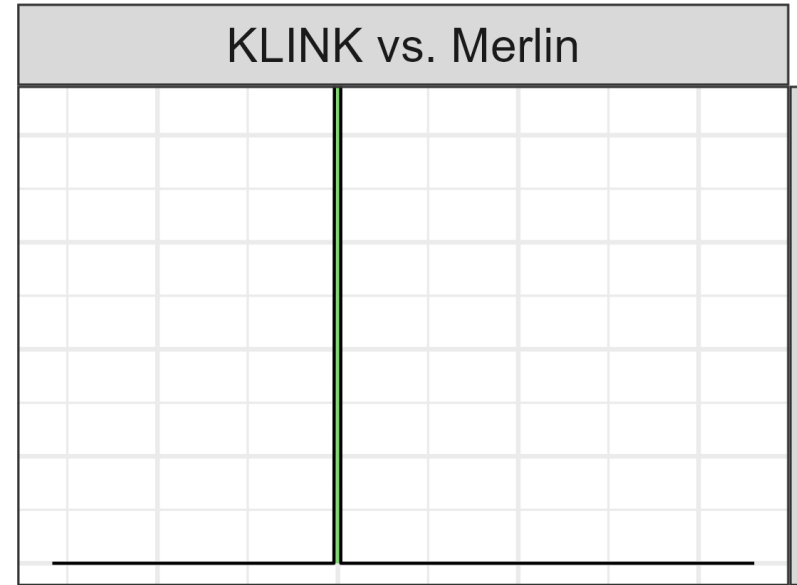
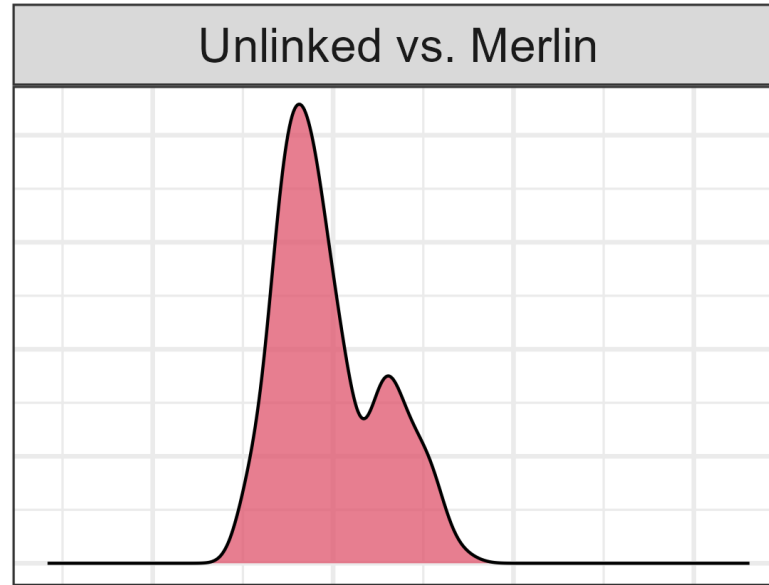
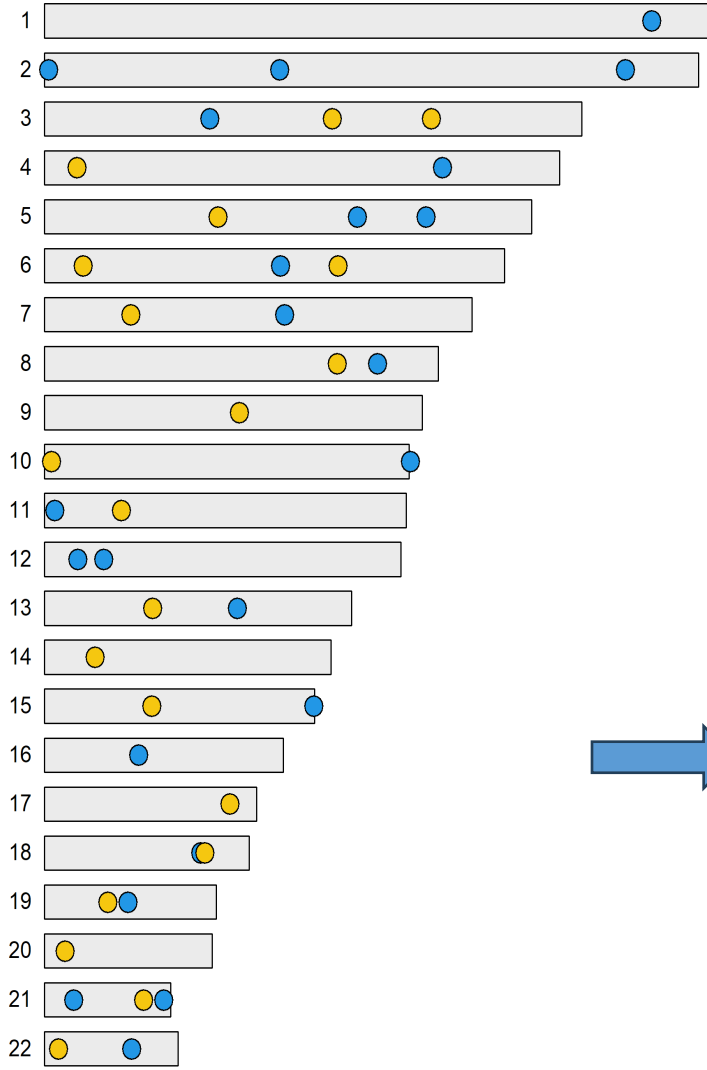


23 markers

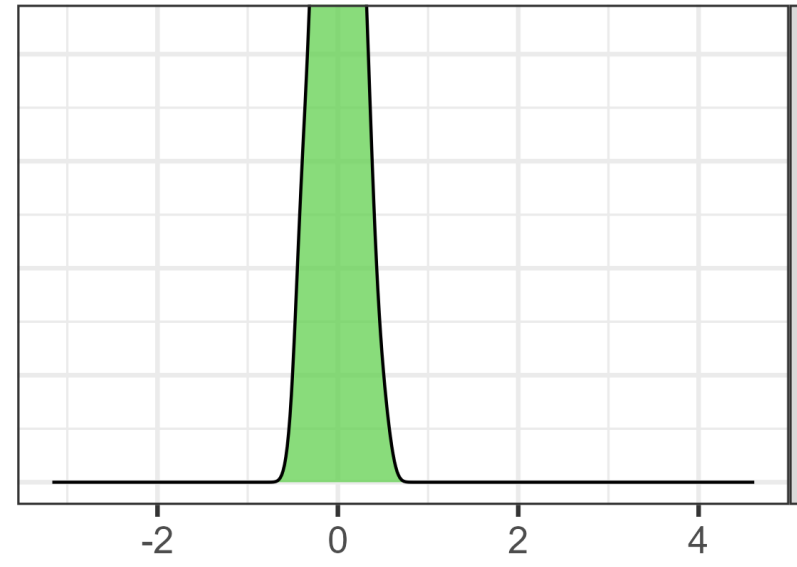
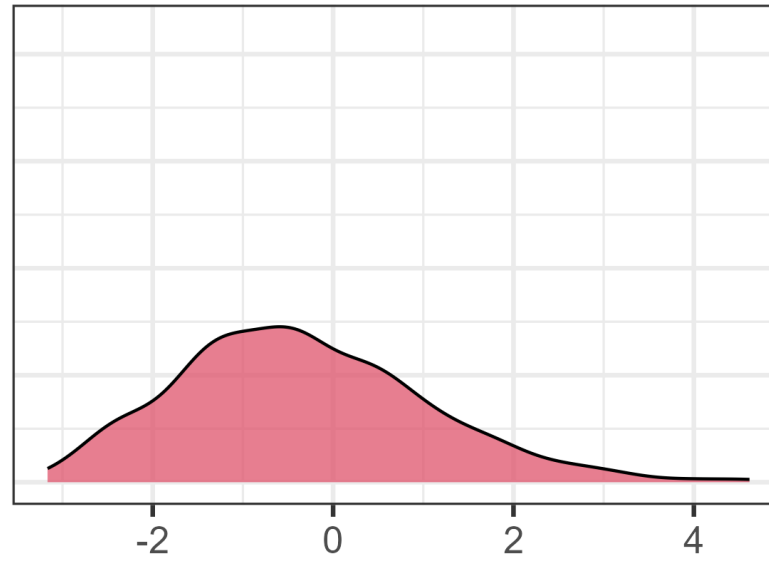


43 markers

log₂ (Fold change)



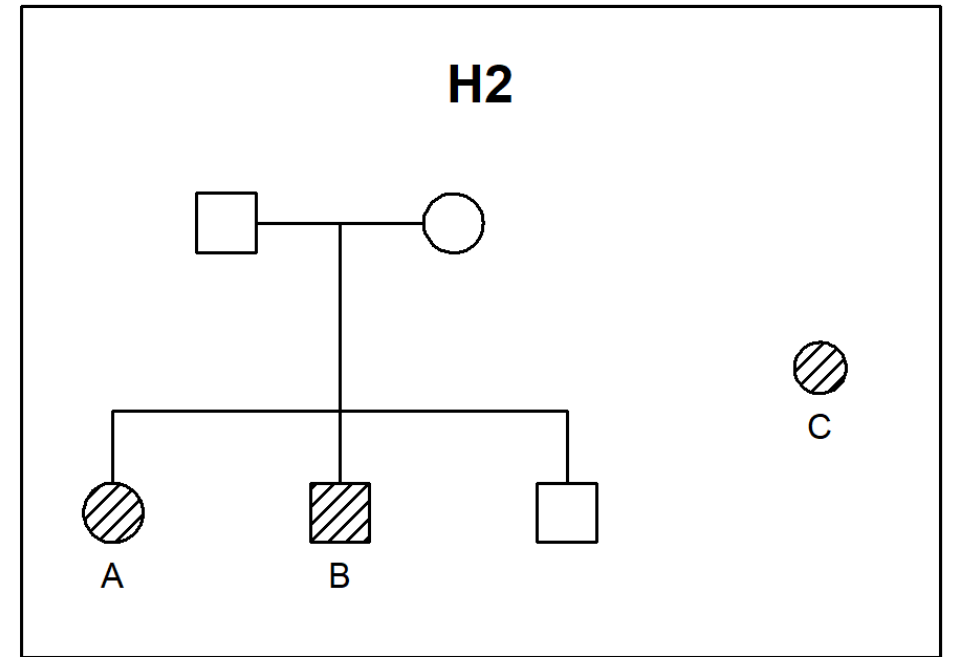
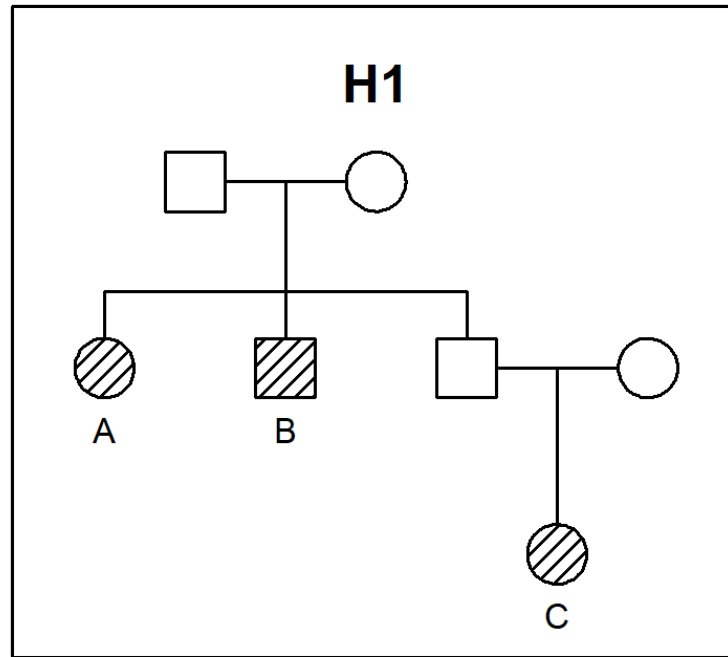
23 markers



43 markers

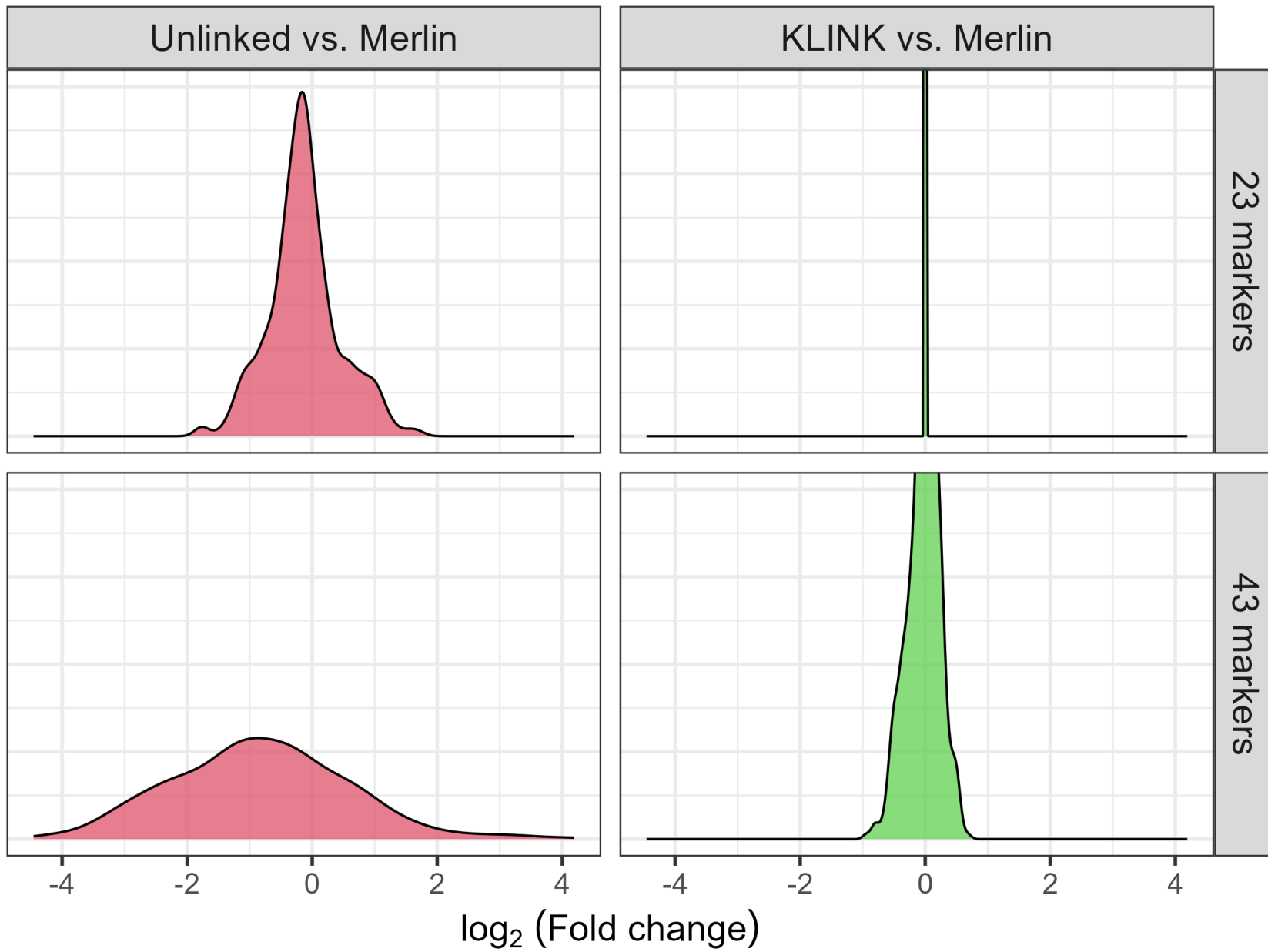
\log_2 (Fold change)

Example 2



Simulation experiment

- 500 profiles for A and B under H1
 - Sims respect linkage
 - Marker sets
 - Fusion6c (23 markers)
 - Fusion6c + SureID (43 markers)
 - Compute LR with
 - No linkage
 - Pairwise linkage (KLINK)
 - Full linkage (Merlin = FamLink2)
- All analyses done in R with the **pedsuite**



Linkage Lab

Playground for linkage enthusiasts



Linkage Lab: Kinship testing with linked markers

<https://magnusdv.shinyapps.io/linkagelab/>

A tool for exploring how linkage affects the LR in kinship testing. Built on the [pedsuite](#). Source code: [GitHub](#).

Comparison
Sibs : half-sibs

Genotypes Simulate

	A1	A2	B1	B2
M1	1	1	1	1
M2	2	2	3	3

Allele frequencies

	1	2	3	4
M1	0,1	0,2	0,3	0,4
M2	0,1	0,2	0,3	0,4

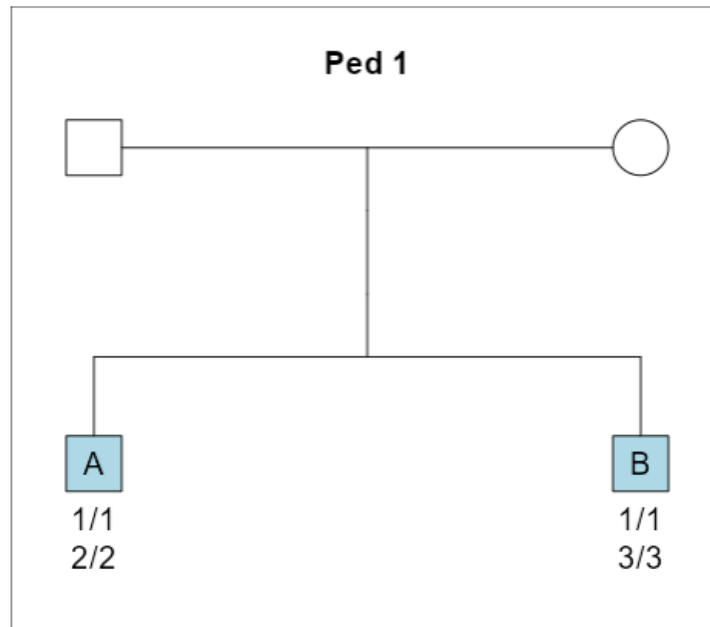
Update

Dist (cM): 0
Rec. rate: 0
Mut. rate: 0

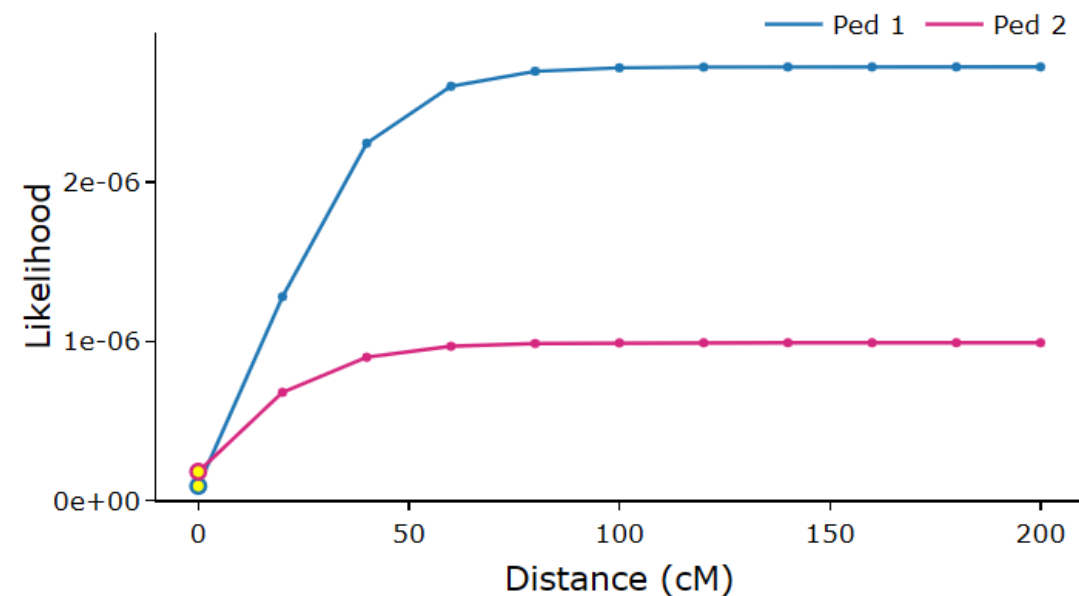
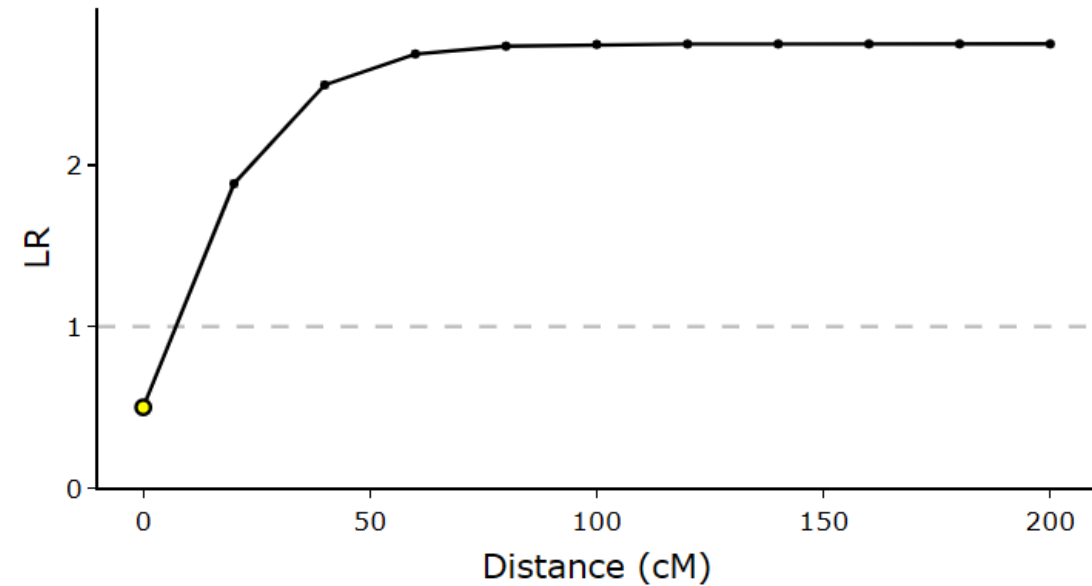
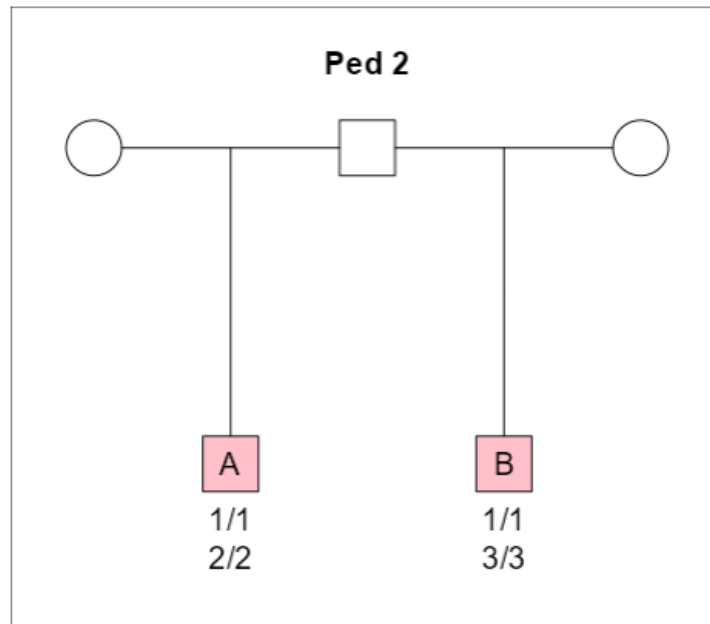
Haldane Kosambi

Plot variable
Distance (cM)

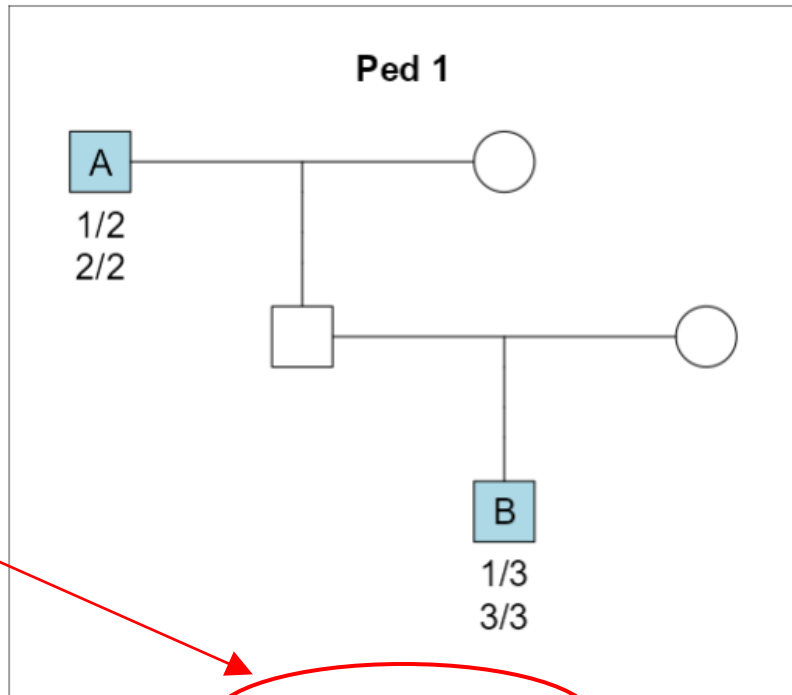
Points: 11
Max: 200



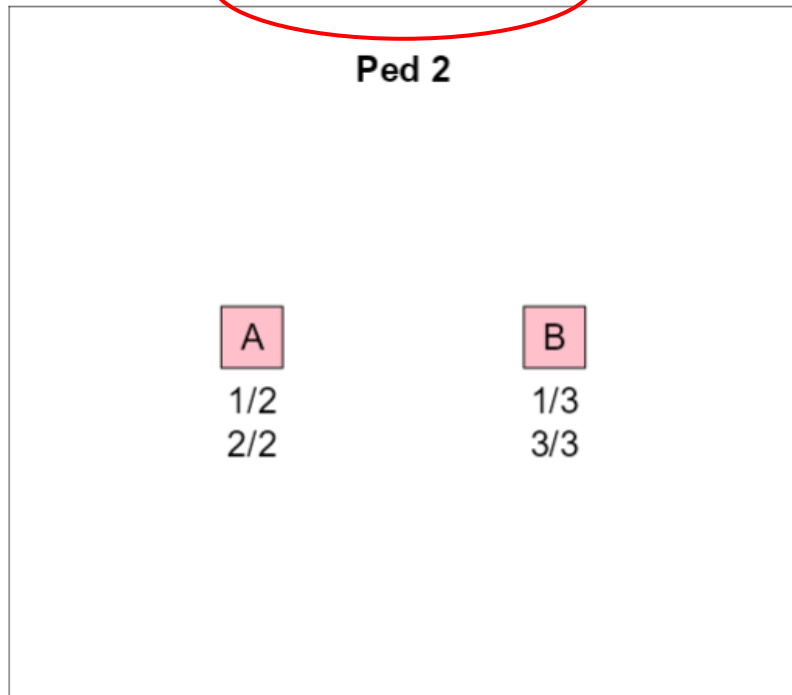
shared alleles: 2 and 0



Important!



shared alleles: 1 and 0



Quiz

Which scenario gives the highest LR?

- A. Markers are linked
- B. Markers are unlinked (independent)

Comparison

Grandparent :
unrelated

Genotypes Simulate

	A1	A2	B1	B2
M1	1	2	1	3
M2	2	2	3	3

Allele frequencies

	1	2	3	4
M1	0,01	0,2	0,3	0,49
M2	0,1	0,2	0,3	0,4

Update

Dist (cM): 0

Rec. rate: 0

Mut. rate: 0

Haldane Kosambi

Plot variable

Distance (cM)

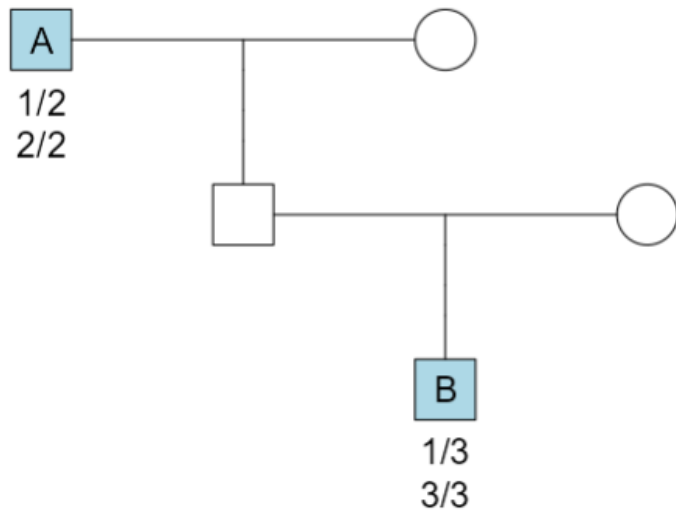
Points

11

Max

200

Ped 1

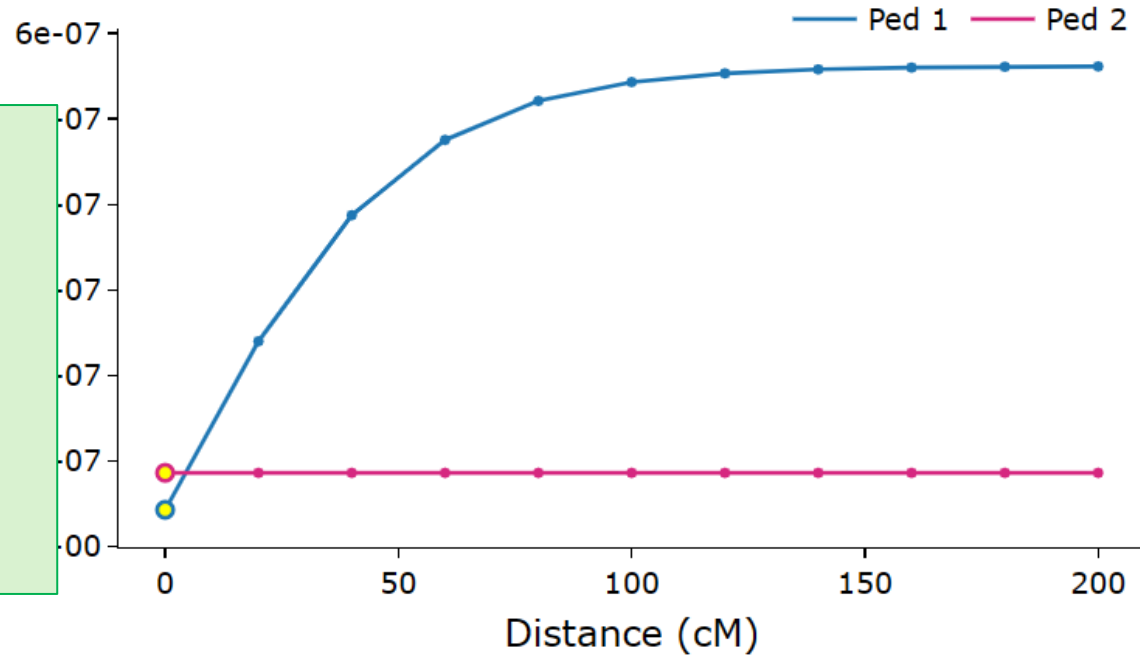
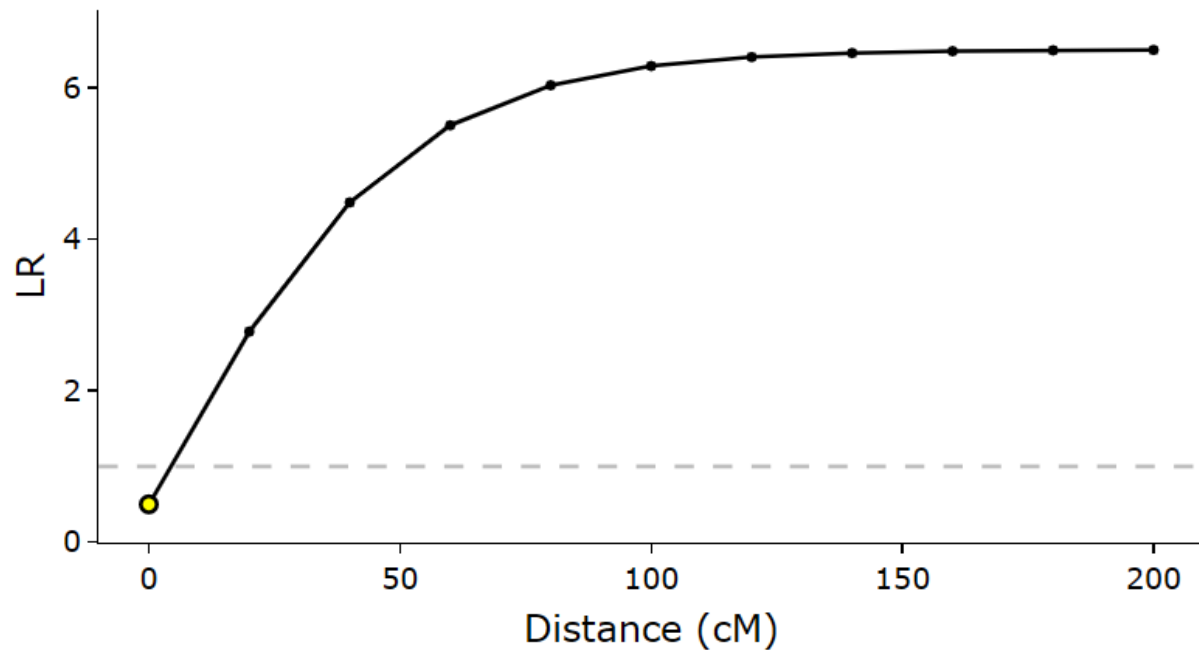


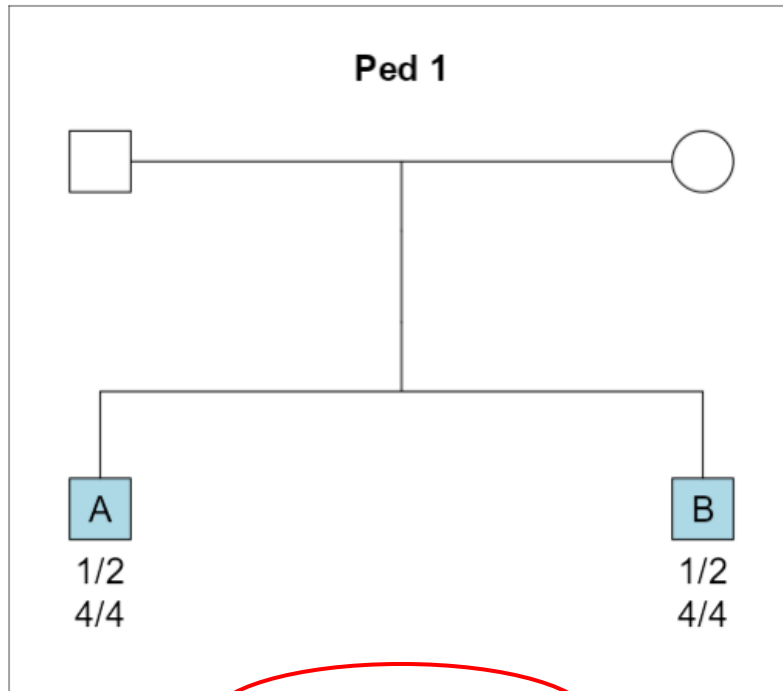
shared alleles: 1 and 0

Ped 2

Logic

- Linkage \rightarrow less recombination
 \rightarrow less likely to change IBD status
- With rare alleles, IBD \approx IBS
- Data: Different IBS at the two markers
- Therefore smaller LR under linkage!





Comparison

Sibs : unrelated

Genotypes

Simulate

	A1	A2	B1	B2
M1	1	2	1	2
M2	4	4	4	4

Allele frequencies

	1	2	3	4
M1	0,1	0,2	0,3	0,4
M2	0,1	0,2	0,3	0,4

Update

Dist (cM): 0

Rec. rate: 0

Mut. rate: 0

Haldane Kosambi

Plot variable

Distance (cM)

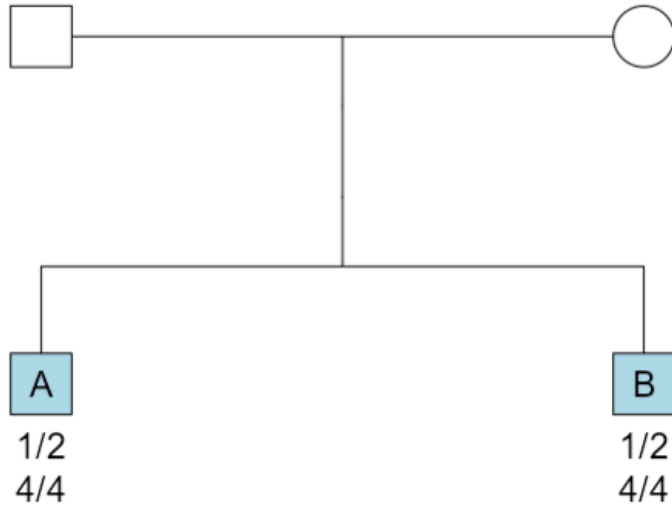
Points

11

Max

200

Ped 1

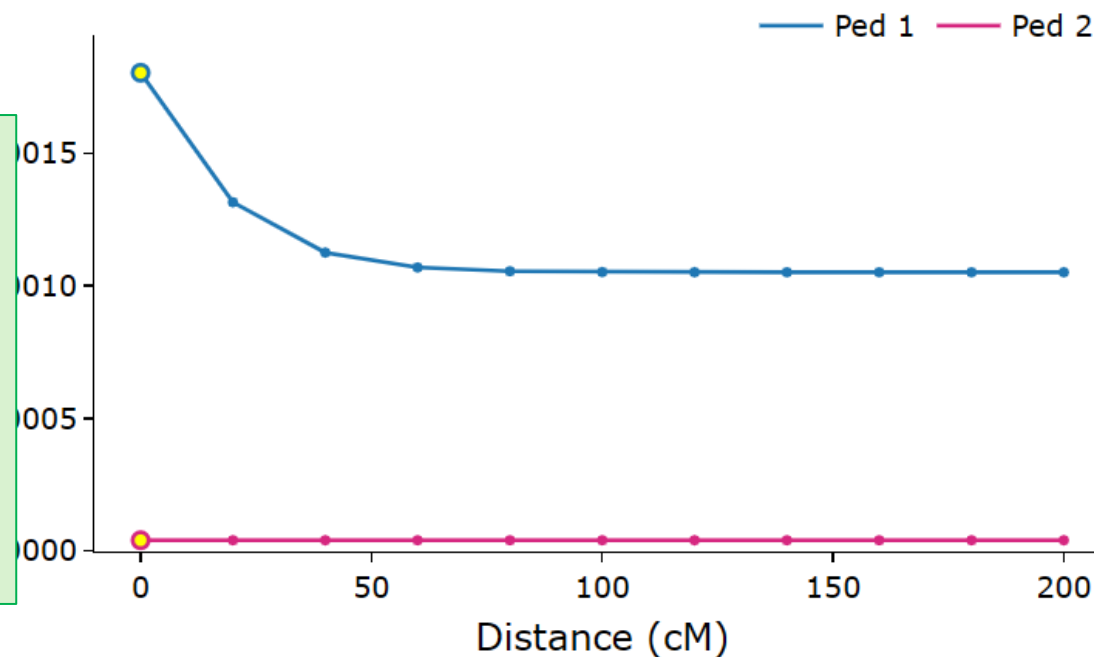
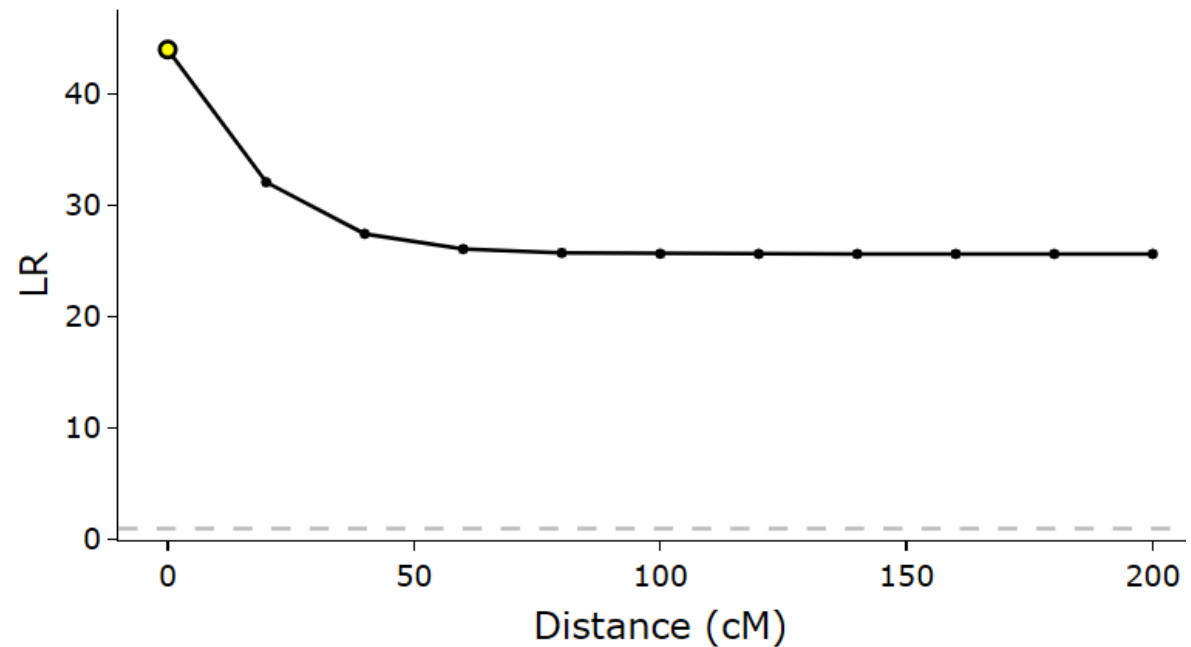


shared alleles: 2 and 2

Ped 2

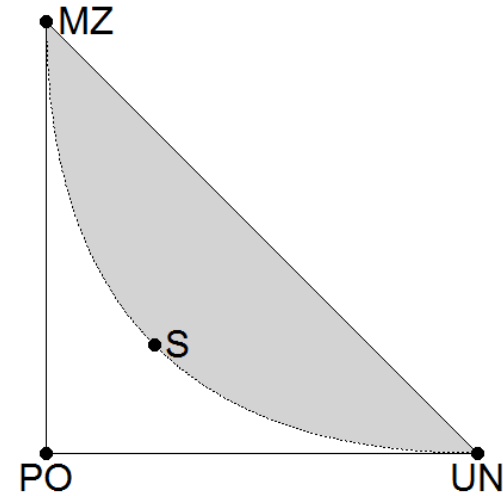
Logic

- Linkage \rightarrow less recombination
 \rightarrow less likely to change IBD status
- With rare alleles, IBD \approx IBS
- Data: Same IBS at the two markers
- Therefore higher LR under linkage!



Summary I

- Linkage is irrelevant in the corners
 - Unrelated
 - Paternity (duo/trio)
 - Monozygotic twins



- **Ignoring linkage** tends to:

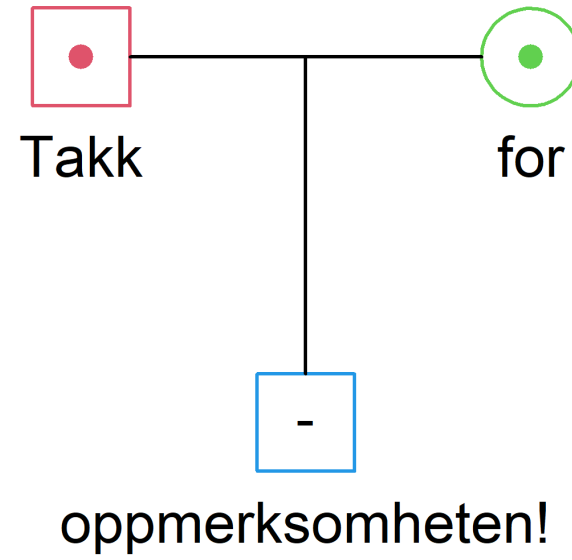
- **Overestimate** the LR if the allele sharing **differs**
- **Underestimate** the LR if the allele sharing is the **same**

These are just rules of thumb. Generally anything can happen!

Summary II

- Standard forensic kinship software (incl. Familias) ignores linkage
- Usually ok with few markers (although LR bias may occur)
- With extended kits (>25 markers) linkage is increasingly important
- Software (both extending Familias):
 - KLINK - good for STRs
 - FamLink - better with SNPs
- Use Linkage Lab to understand how linkage affects your data!





```
x = pedtools::nuclearPed()
plot(x, col = 2:4, carrier = 1:2, textInside = c(`3` = "-"),
     labs = c(`Takk` = 1, `for` = 2, `oppmerksomheten!` = 3))
```