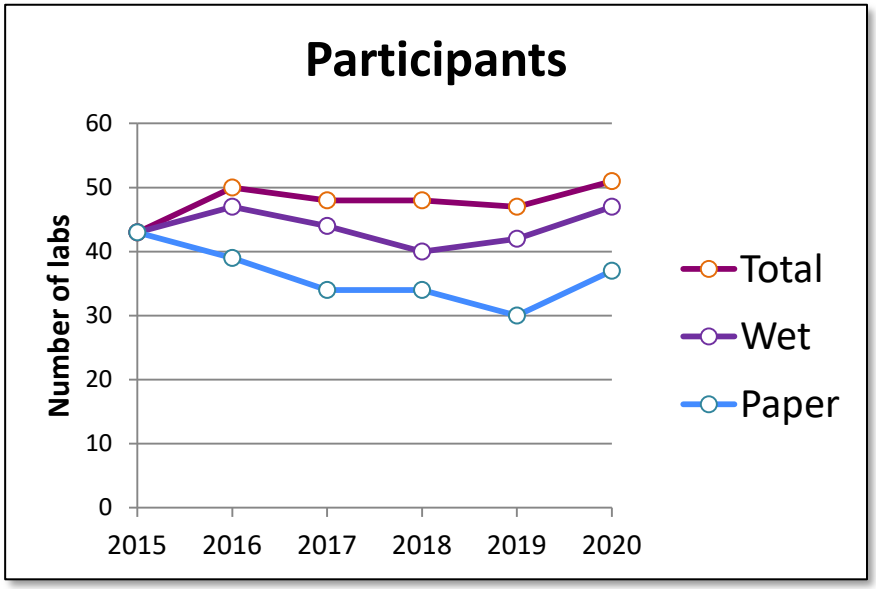
ESWG - Proficiency test 2021

Daniel Kling – Head Organizer

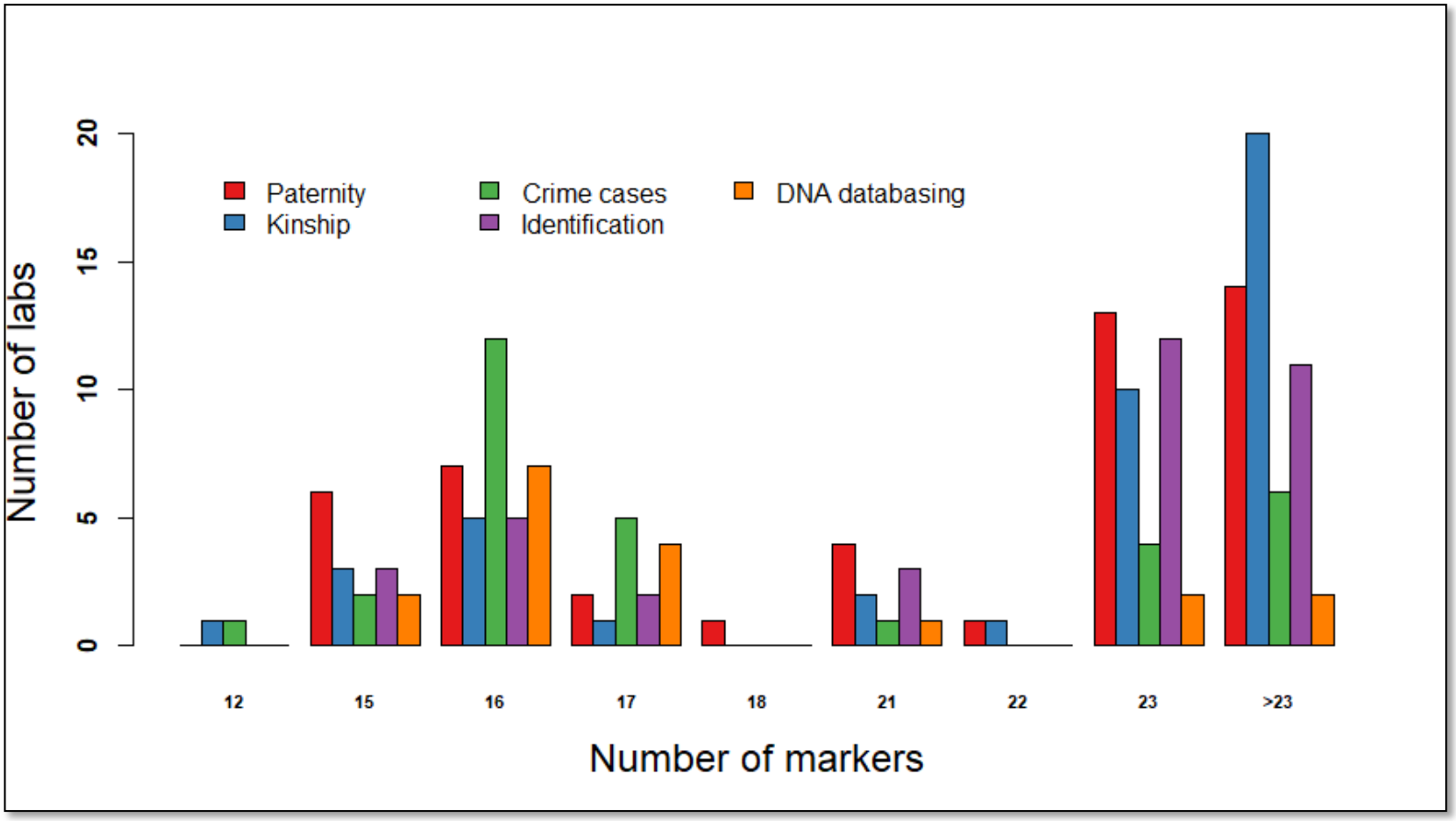
daniel.kling@rmv.se

Summary

- 51 labs participated
- 37 completed paper challenge
- 47 completed wet exercise

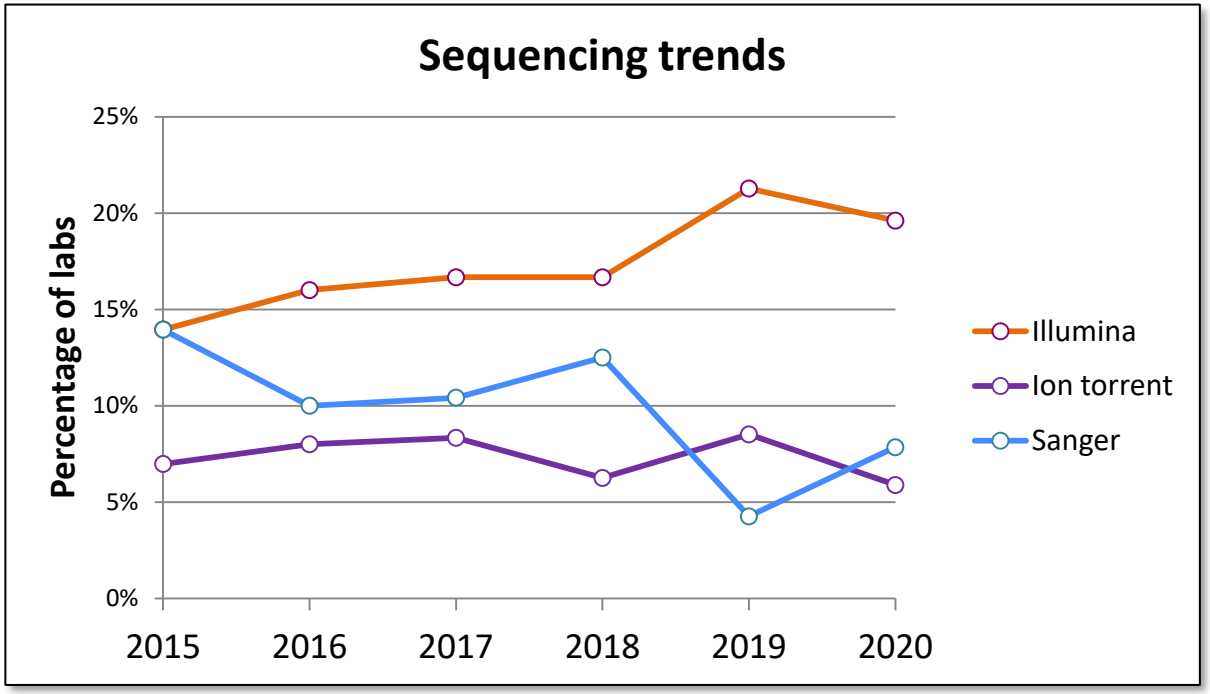


Questionnaire – Markers used

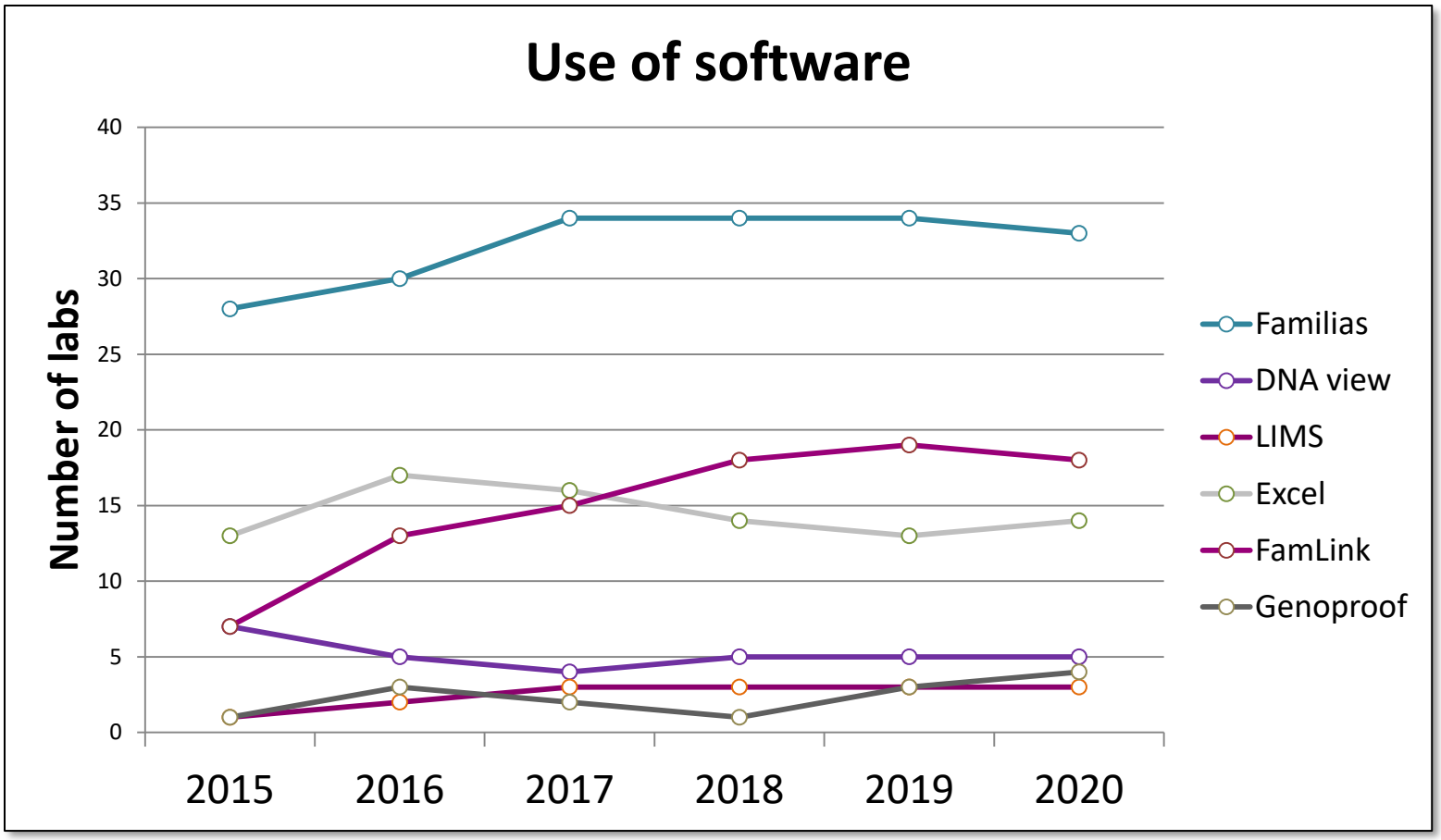


Questionnaire – Sequencing trends

- 18 labs (35%) own sequencing instrument. 2 planning to buy



Questionnaire – Software trends



Questionnaire – Linked markers

Not accounted for: 10

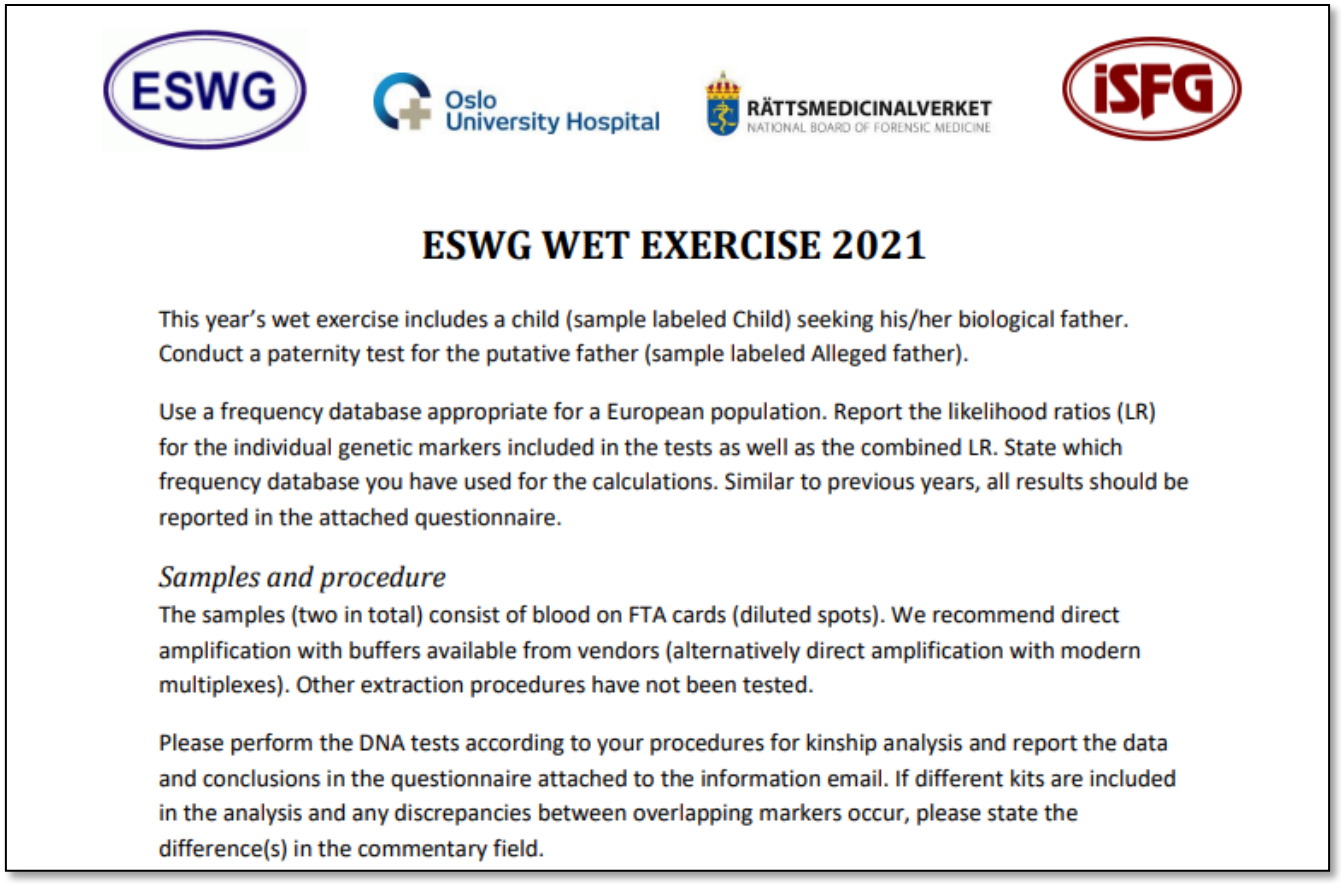
Not used: 15

Exclude one: 15

Accounts for: 11

WET EXERCISE

Wet exercise - Background



ESWG

Oslo University Hospital

RÄTTSMEDICINALVERKET
NATIONAL BOARD OF FORENSIC MEDICINE

ISFG

ESWG WET EXERCISE 2021

This year's wet exercise includes a child (sample labeled Child) seeking his/her biological father. Conduct a paternity test for the putative father (sample labeled Alleged father).

Use a frequency database appropriate for a European population. Report the likelihood ratios (LR) for the individual genetic markers included in the tests as well as the combined LR. State which frequency database you have used for the calculations. Similar to previous years, all results should be reported in the attached questionnaire.

Samples and procedure

The samples (two in total) consist of blood on FTA cards (diluted spots). We recommend direct amplification with buffers available from vendors (alternatively direct amplification with modern multiplexes). Other extraction procedures have not been tested.

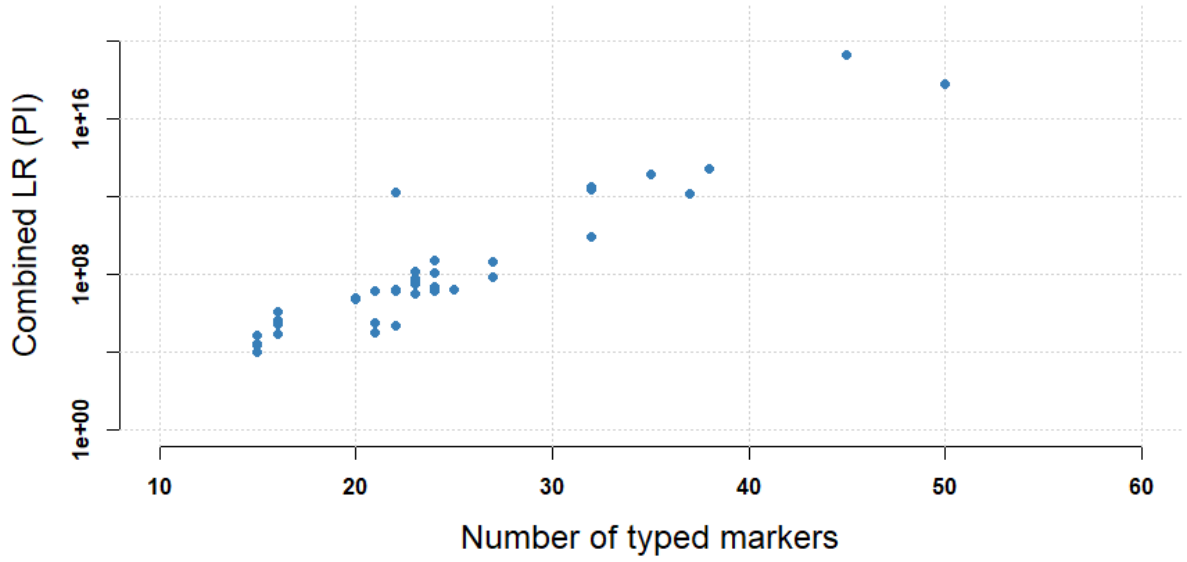
Please perform the DNA tests according to your procedures for kinship analysis and report the data and conclusions in the questionnaire attached to the information email. If different kits are included in the analysis and any discrepancies between overlapping markers occur, please state the difference(s) in the commentary field.

Wet exercise - Summary

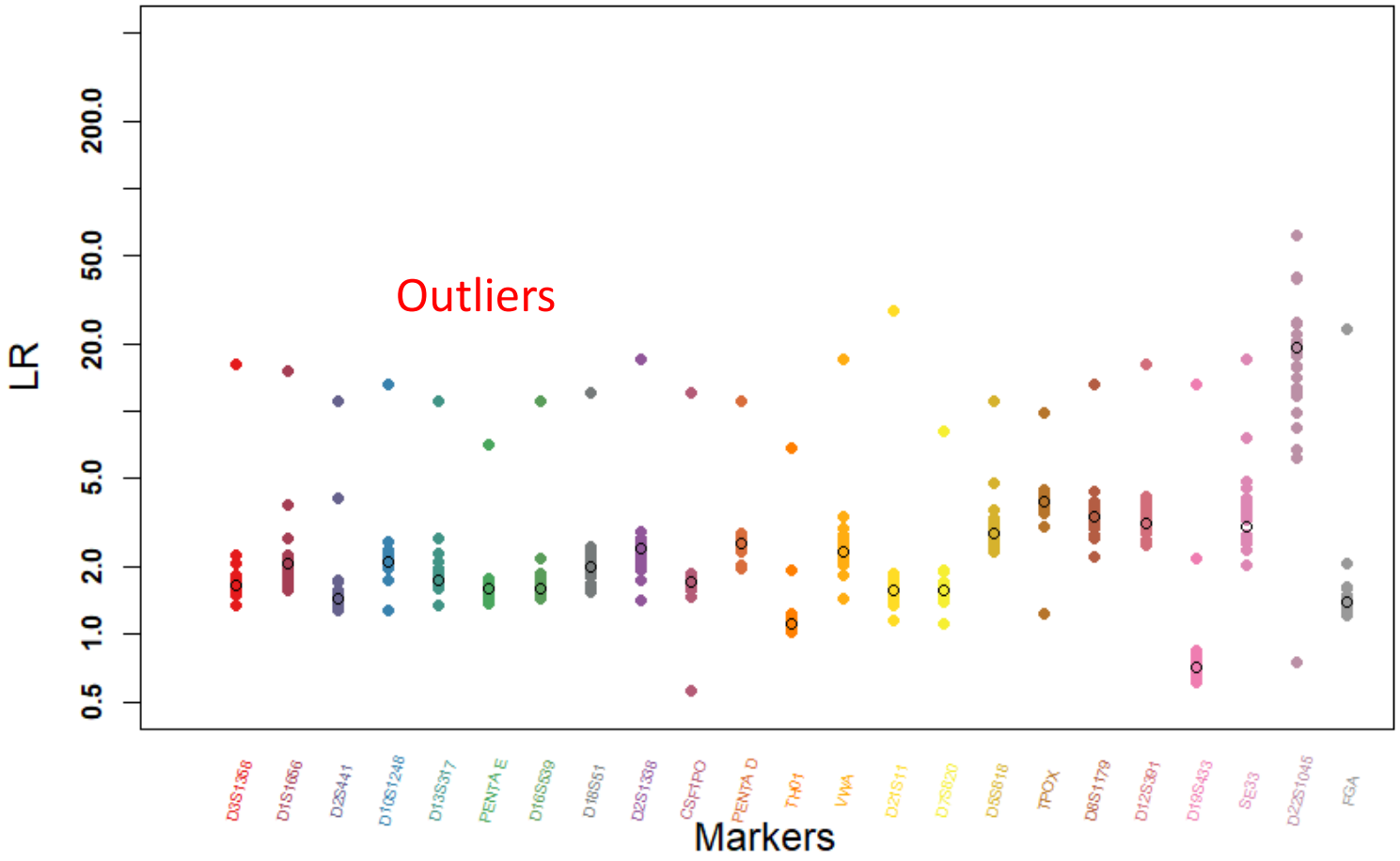
- Overall very concordant results (despite the use of potentially different databases)
- 49 labs participated (47 submitted results)
- Consult the Excel summary for details
- For the wet exercise some labs' results have been highlighted (red or orange) which indicates a result that deviates. Certificates will still be issued.

Wet exercise

- A single alleged father
- A single lab reported SNP markers
- Marker D22S1045 displayed greatest variation (6-40)



Wet exercise – per marker LR variation



PAPER CHALLENGE

Paper challenge - Background

- A mysterious case
- Four children
- Generate pedigrees

Paper challenge – Setup

ESWG Oslo University Hospital RÄTTSMEDICINALVERKET NATIONAL BOARD OF FORENSIC MEDICINE iSFG

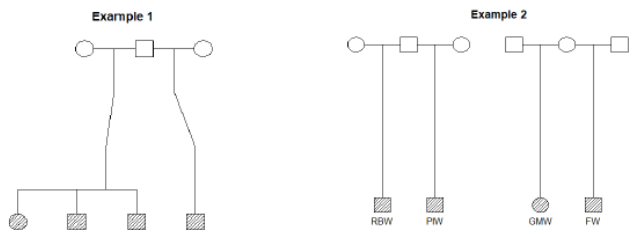
ESWG PAPER CHALLENGE 2021

This year's paper challenge consists of a single exercise. In order to obtain the certificate, participants have to submit results. All data is given as files at https://familias.name/ESWG/ESWG2021_paperchallenge.zip in addition to some details given directly in the cases. Please fill out all answers in the supplied Excel questionnaire.

Case – Mystery at the Burrow

In the countryside lay a peculiar old house adorned with chimneys, stretched vertically to accommodate its many inhabitants. According to history there once lived a famous family with more children and grandchildren then one can count. However, recent allegations have been made as to whether or not some of the children were indeed siblings or not. We were able to obtain DNA data from four of the children: GMW, RBW, PIW and FW. You are asked to,

a) List all possible pedigrees for these four children. That is, given that they can be either full, half siblings or unrelated, provide the number of all potential combinations between the four. For example, the pedigrees below list two such combinations. We refer to the combination where all four individuals are unrelated as the null hypothesis..



Paper challenge – Part a)

- List all possible combinations with the following conditions
  1. Four children (none of them can be parents)
  2. Any number of parents
  3. No inbreeding etc
  4. Essentially all sibling combinations
- Essentially a combinatorial problem
  1. Software to generate pedigrees (only non-redundant)
  2. We can work out the number mathematically

Demonstration in Familias

Paper challenge – Part a)

- Familias will give you 225 unique pedigrees (maternal/paternal half sibs are considered separately). 6561 if redundancy is not considered.
- 9 labs reported 225, other numbers are all different
- Stirling and Bell numbers will give you the same

Let's give it a try!

Paper challenge – Part a)

- Stirling number of the second kind S(n,k) will give you how many ways to combine *n* objects into *k* sets.
- We can use tables or formulas, see https://en.wikipedia.org/wiki/Stirling_numbers_of_the_second_kind

k is the number of sets (fathers/mothers)

n is the number of children

n \ k	0	1	2	3	4	5
0	1					
1	0	1				
2	0	1	1			
3	0	1	3	1		
4	0	1	7	6	1	
5	0	1	15	25	10	1

Paper challenge – Part a)

- *n* means the number of children, i.e. 4
- *k* means the number of paternal or maternal relations. So k=1 means all children are unrelated (a single father) and k=4 means they are all paternally/maternally related

k is the number of sets (fathers/mothers)

n is the number of children

n \ *k*	0	1	2	3	4	5
0	1					
1	0	1				
2	0	1	1			
3	0	1	3	1		
4	0	1	7	6	1	
5	0	1	15	25	10	1

Paper challenge – Part a)

S(4,1)=1, There is 1 way to partition 4 children among 1 father. This is the pedigree where one father has four children.

S(4,2)=7, There are 7 ways to partition 4 children among 2 fathers. These are the pedigrees where 1 father has 3 children and the other father has 1 child or each father has 2 children. See below.

S(4,3)=6, There are 6 ways to partition 4 children among 3 fathers. These are the pedigrees where 1 father has 2 children and two fathers each have one child. See below.

S(4,4)=1, There is 1 way to partition 4 children among 4 fathers. These are the pedigrees where each of the four fathers has one child.

k is the number of sets (fathers/mothers)

n is the number of children

n \ *k*	0	1	2	3	4	5
0	1					
1	0	1				
2	0	1	1			
3	0	1	3	1		
4	0	1	7	6	1	
5	0	1	15	25	10	1

Paper challenge – Part a)

- Next, Bell numbers (B_n) are the sum for a particular *n* of all Stirling numbers.
- So B_4=1+7+6+1=15 in the table below

k is the number of sets (relations)

n is the number of children

n \ *k*	0	1	2	3	4	5
0	1					
1	0	1				
2	0	1	1			
3	0	1	3	1		
4	0	1	7	6	1	
5	0	1	15	25	10	1

Paper challenge – Part a)

- To get the total number of pedigrees we multiply the maternal and paternal number of pedigrees, 15x15=225
- Fewer if we do not consider maternal and paternal half siblings separately.
- Tricky, but may be useful!

We will next consider subsets of these pedigrees!

Paper challenge – Part b)

b) Discuss approaches to resolve the case. What approach would your lab take to solve the case? Approaches might include, but not limited to

1) Pairwise – Samples are compared in a pairwise fashion and a relationship is considered as confirmed or excluded if the comparison exceeds some threshold.
2) Stepwise – Sample are compared both in a pairwise fashion and in a joint fashion. Starting with pairwise comparisons, where a relationship is considered as confirmed or excluded if it exceeds some threshold. The comparisons are iterated through the inclusion of confirmed matches, until no comparison exceeds the threshold.
3) Joint Bayesian – All relevant pedigrees listed in a) are combined and computations performed for all these.

Paper challenge – Part b)

- 9 labs would do the pairwise approach
- 11 labs would use the stepwise approach
- 16 labs would do the joint Bayesian

Paper challenge – Part b)

Example: Consider three potential full siblings (S1,S2 and S3), inclusion threshold=100 (or 99%)

LR(S1 and S2)=10
LR(S1 and S3)=100
LR(S2 and S3)=10
LR(S1, S2 and S3)=20000

Pairwise approach would conclude that S1 and S3 are full siblings, S2 is inconclusive.

Joint Bayesian approach would conclude that all are full siblings, since LR(S1,S2 and S3)/LR(S1 and S3)=20000/100=200 or more correctly we would use posteriors.

Pr(S1,S2 and S3)=20000/(20000+10+10+100)=99.4%

Paper challenge – Part c)

c) Using the DNA data provided below, calculate and report the LR comparing the combinations where all individuals are full siblings versus the null hypothesis, see a). What conclusions can be drawn from this LR?

DNA data is given below and in the online files.

Marker	GMW	RBW	PIW	FW
CSF1PO	11,13	12,11	12,11	12,11
D13S317	11,12	11,12	11,11	11,12
D16S539	12,14	12,14	11,14	12,12
D18S51	14,13	14,13	14,13	14,13
D19S433	14,13	13,13	14,13	13,13
D21S11	31,30	28,30	31,31.2	28,30
D2S1338	23,19	25,19	25,23	25,19
D3S1358	17,16	16,16	17,13	17,16
D5S818	11,11	11,11	11,12	11,11
D7S820	11,9	8,12	11,9	8,12

Paper challenge – Part c)

- Expected LR=4E+27 -> Extremely strong evidence in favor of full siblings
- A possible mutation at SE33, consistent with a one step event

SE33	4.85438133	14, 27.2	14, 27.2	20, 27.2	21, 27.2

Paper challenge – Part d)

d) Additional information is given stating that GMW, RBW and FW share the same mother. Use this information to reduce the space of possible pedigrees. How many pedigrees remain?

- Can be worked out in Familias, 30 unique pedigrees
- 11 labs reported 30 pedigrees
- Can be worked out using Stirling and Bell numbers!

Recall, there are still 15 combinations where they can be paternally related, but now only 2 ways they can be maternally related. So in total 15x2=30 pedigrees

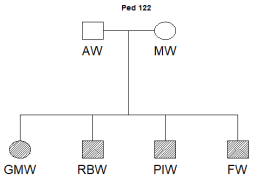
Paper challenge – Part e)

e) Using the provided DNA data and the complete list of pedigrees listed in **d)**, compute the likelihoods and posterior probabilities for all combinations. Report the top 10 results and your verbal conclusion in the case.

Demonstration in Familias

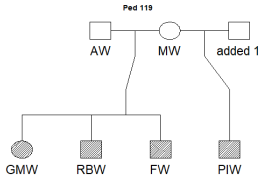
Paper challenge – Part e)

- Top 3 results amounts to 0.999 (99.9%) of the posterior probabilty
- 24 labs reported full siblings as the most likely

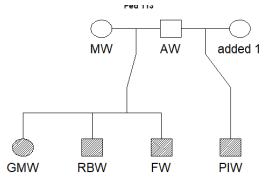


LR=3.72E+17, posterior=0.86

Identical LRs



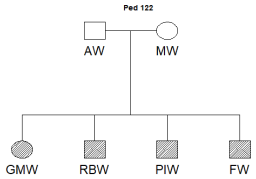
LR=2.99E+16, posterior=0.069



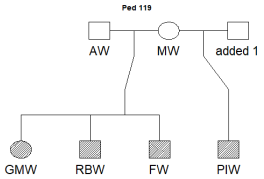
LR=2.99E+16 , posterior=0.069

Paper challenge – Part e)

- If we decide that half siblings should not be distuinguised (maternal from paternal)



LR=3.72E+17, posterior=0.92



LR=2.99E+16, posterior=0.074

Paper challenge – Summary

- Complex case
- Generate pedigrees
- Evaluate different approach to multiple pedigree testing
- Posterior versus LR
- Mutation(!)
- Video will be available through https://familias.name/ESWG/

Proficiency test – Future

- Not determined who will organize next – ESWG board will decide

ESWG - Proficiency test 2021

Daniel Kling – Head Organizer

daniel.kling@rmv.se