ESWG - Proficiency test 2024

Daniel Kling – Head Organizer

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What is it all about?

- Accreditation generally require proficiency testing

- Focus on various aspects of kinship testing

- ESWG currently arranges the test in collaboration with OUS (Norway) and RMV (Sweden)

- Decided each year what happens next

- Two parts (three parts this year)
  - Wet exercise (paternity/maternity case)
  - Paper challenge (one or more challenging cases, e.g. Siblings, mutations etc)
  - SNP exercise (New 2024! – presented separately)

What is it all about?

- A few weeks work each year to create/dispatch/gather/summarize

- Result is a report (usually Powerpoint->pdf)

- A certificate is given if complete the exercise

- Incorrect data in submissions are handled internally by labs

- Questionnaire summarizing methods/kits used

Certificates

- One for each proficiency test
  - Paper challenge (complete a))
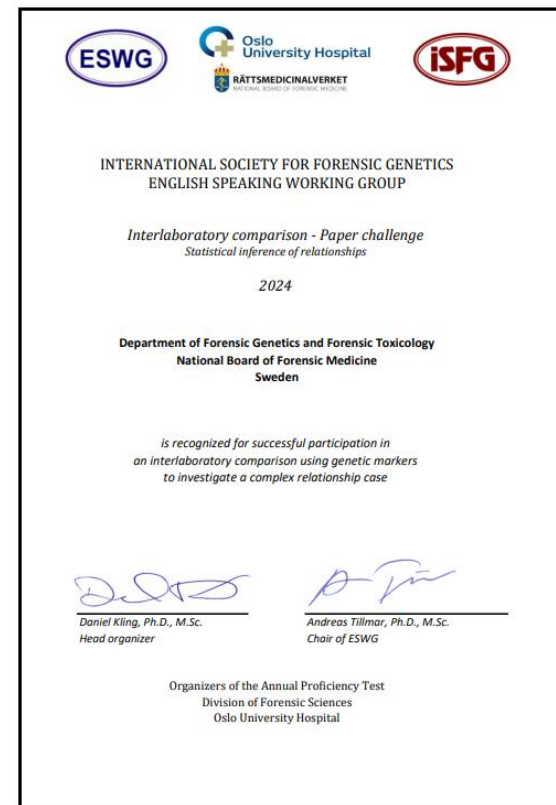
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- One for each proficiency test
  - Paper challenge (complete a))
  - Wet exercise (with/without rem

Certificates

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    - Paper challenge (complete a))
    - Wet exercise (with/without remarks)
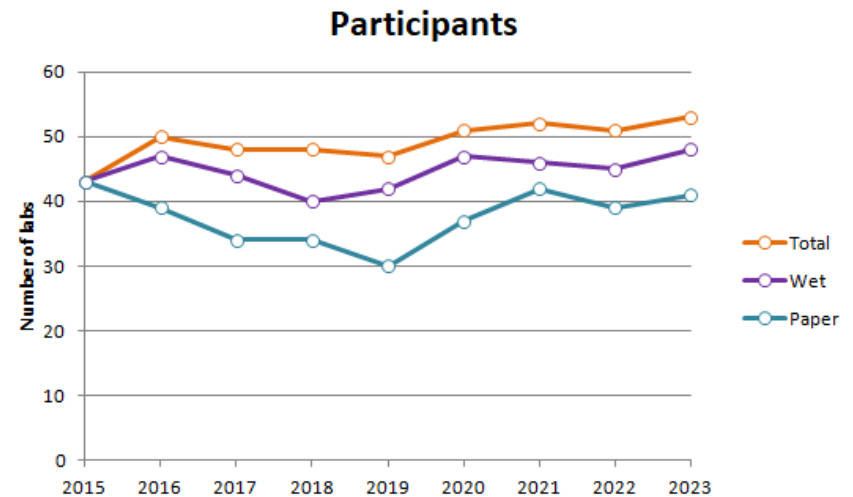   - SNP exercise (given separately)

Survey

- Feedback

- Accreditation – ISO17043

  - No we are not accredited

  - We fulfill the criteria but $$$

https://forms.gle/6b7VYNJLB8muAGPYA

Summary

- Summarizing statistics for 2024
- <u>58</u> labs participated (some SNP only)
- <u>41</u> completed paper challenge
- <u>48</u> completed wet exercise
- <u>13</u> signed up for SNP exercise

Participants

Summary

- MPS data (SNP exercise)

  FORCE (5 labs), Forenseq Signature (2 labs), Identity SNP (Thermo), Microarrays (GSA and custom), Custom hybridization capture (1.3M+ SNPs)
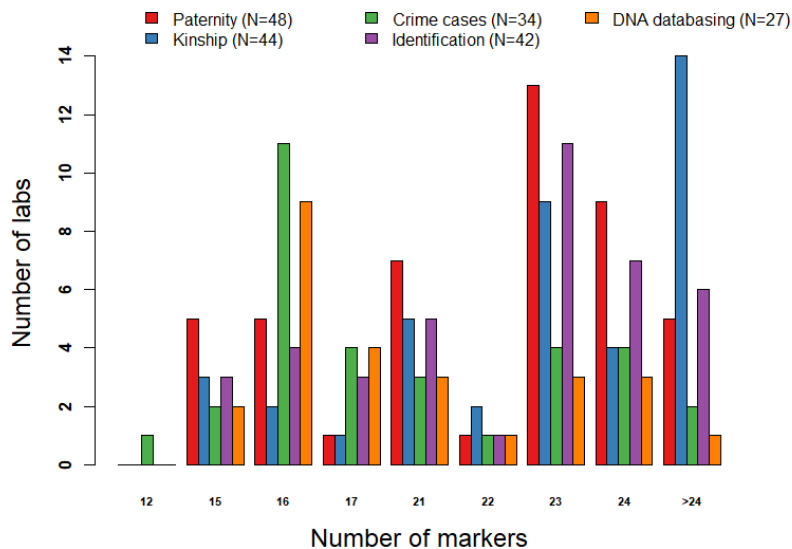
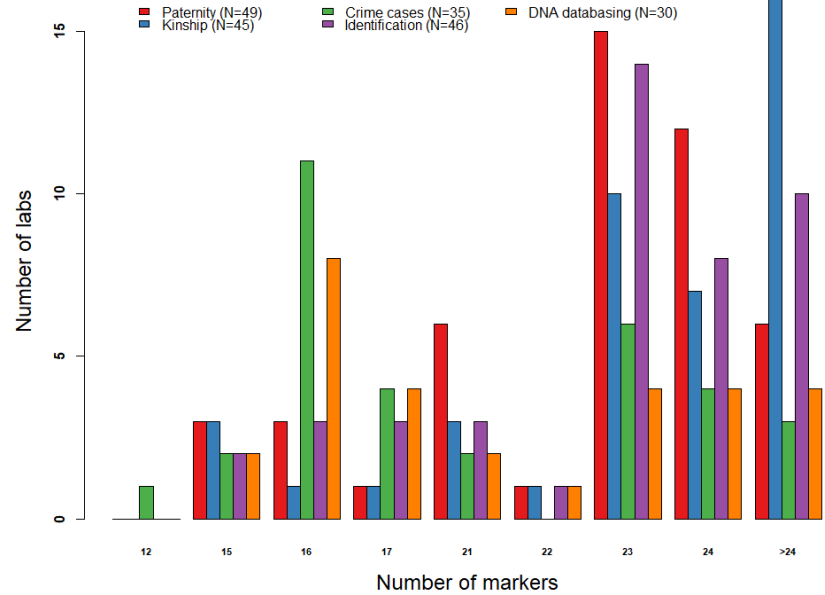 <u>Initial results suggest very high concordance (>99.9%)!</u>

  <u>Struggles</u>
  i.   13 labs -> 13 ways to present genotypes
  ii.   rsIDs can change
  iii. hg19 vs hg38
  iv.   Forward vs reverse reporting

Oslo
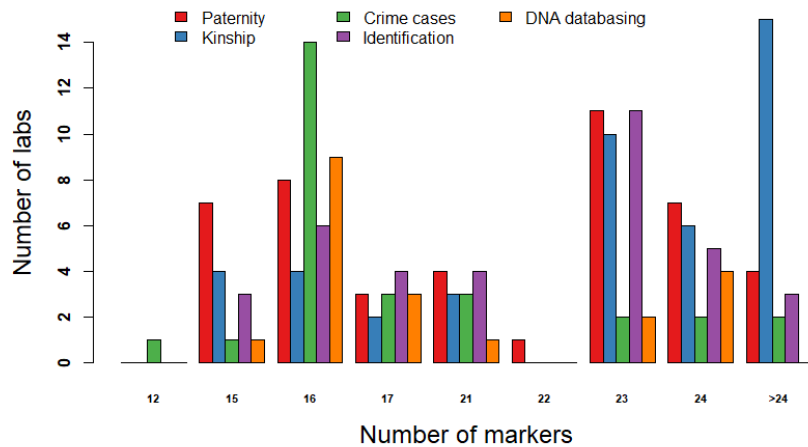University Hospital

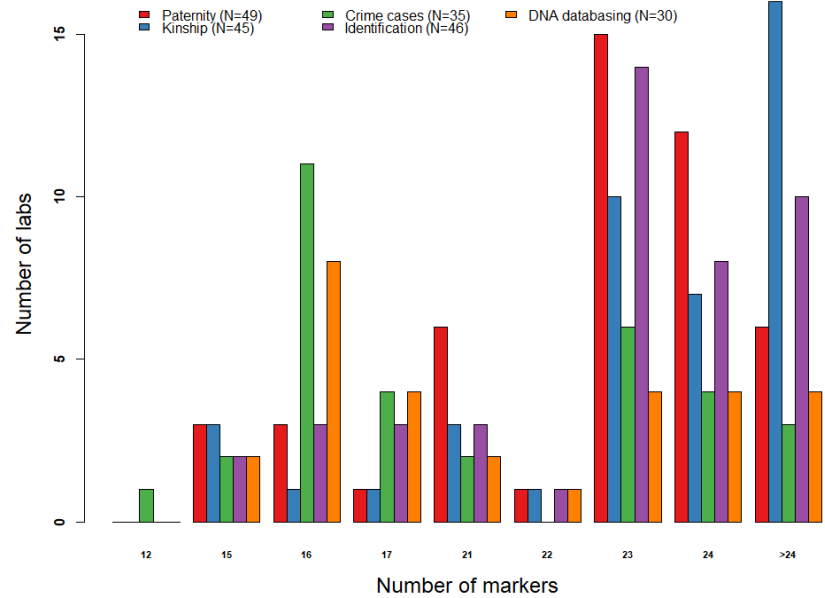# Questionnaire – Markers used

2022

2023

Questionnaire – Markers used

2019
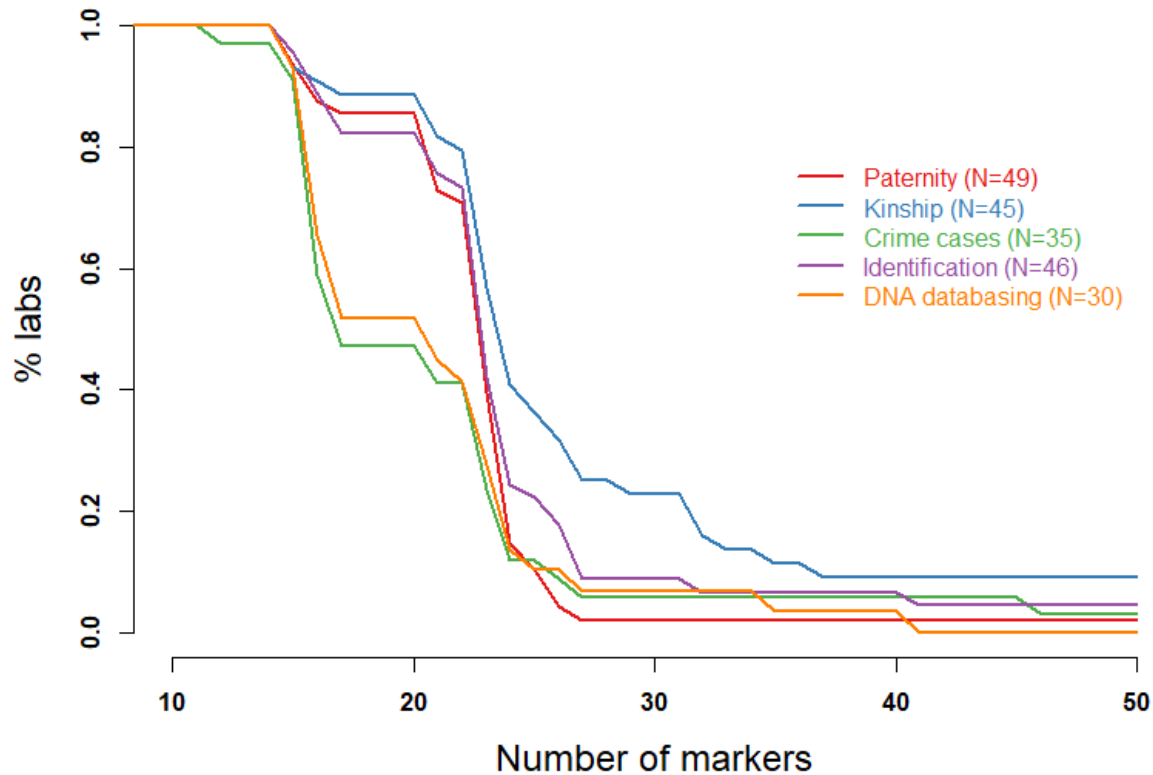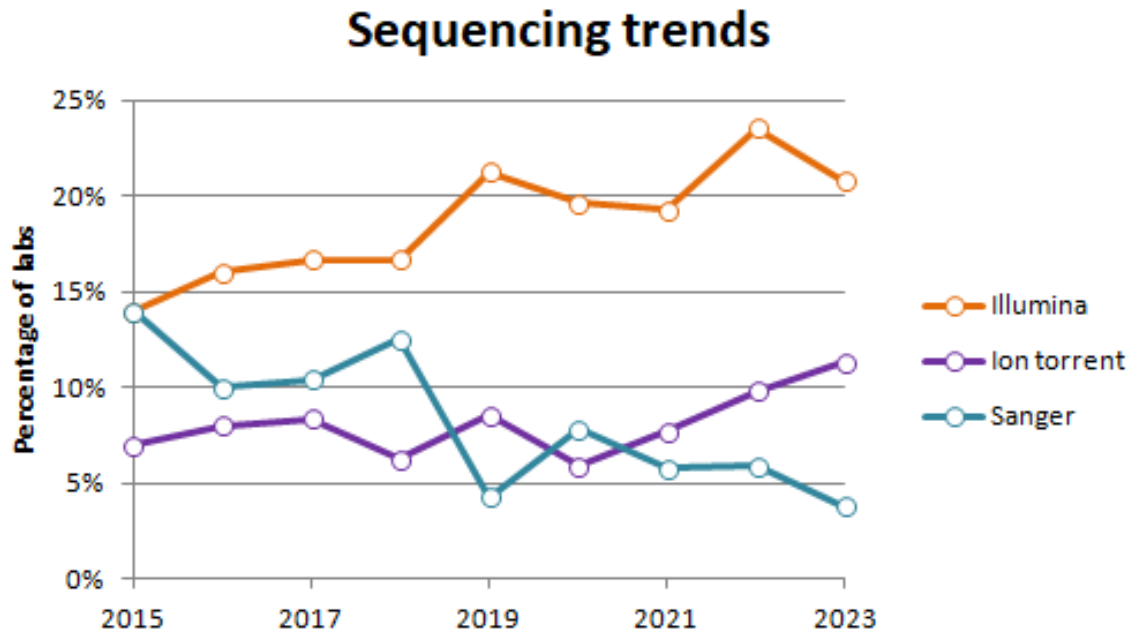
2023

Questionnaire – Markers used

2023

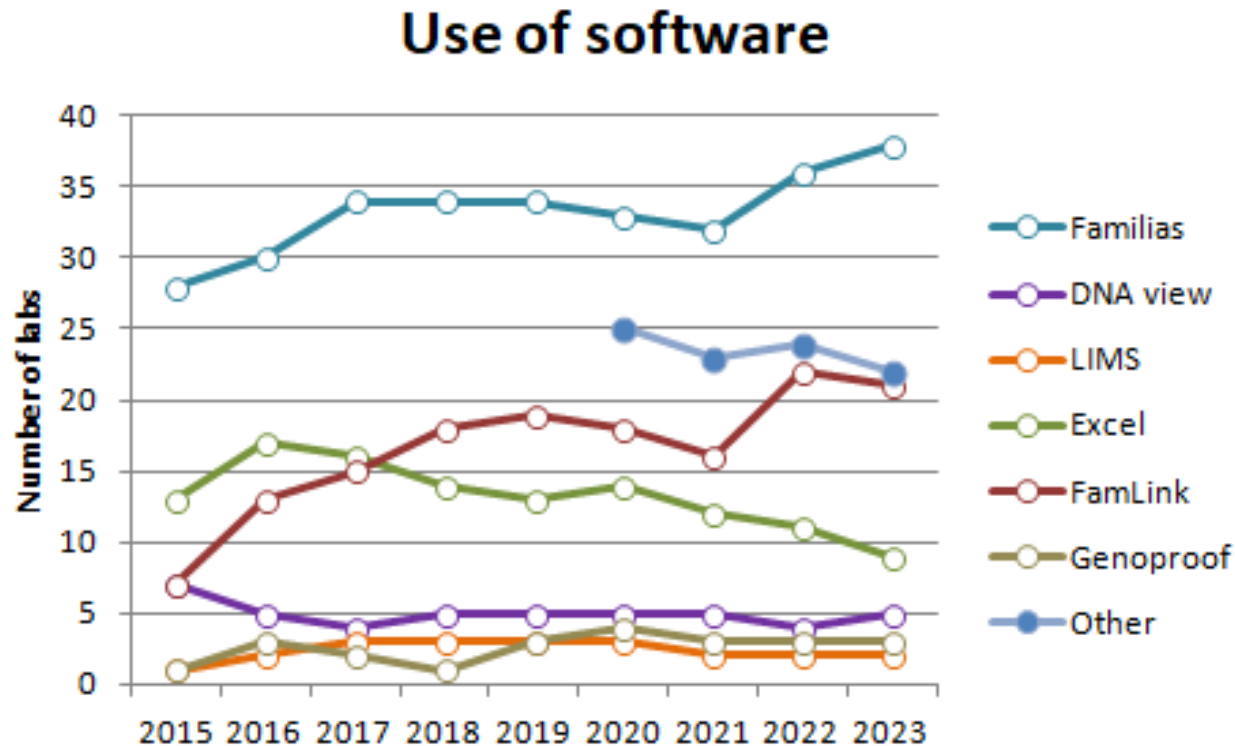# Questionnaire – Sequencing trends

- 19 labs (36%) performs sequencing. Same number as last year

Sequencing trends

Questionnaire – Software trends

Other include DBLR. EuroForMix. STRmix and more

Questionnaire – Subpopulation effects

Never includes: 23 (2022: 21)

Always include: 15 (2022: 15)

When it is known: 15 (2022: 16)

Roughly 60% accounts for subpopulation effects

Questionnaire – Linked markers

Not accounted for: 10 (2021-2022: 10 -> 11)

Not used: 14 (2021-2022: 14 -> 13)

Exclude one: 17 (2021-2022: 16 -> 13)

Accounts for: 14 (2021-2022: 9 -> 13)

Increase in labs that exclude one marker

WET EXERCISE

Wet exercise - Background

ESWG WET EXERCISE 2024

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 electronic spreadsheet 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. Reach out to Daniel.kling@rmv.se for any assistance or questions.

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 comment field.

Due date
The due date is August 31th, results submitted after this deadline may be dismissed.

Same procedure as last years – diluted blood on FTA cards -> works well on direct amplication. <u>Some labs have problems with extraction</u>

Wet exercise - Summary

➢ A few discordant typing results

➢ Overall very concordant statistical results (despite the use of potentially different databases)

➢ 48 labs participated

➢ 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 but with remarks for red results.

| Combined LR (reported)		8,19E+06	5,60E+12	2,20E+10	4,93E+06						6,24E+14	1,62E+10					9,08E+09	1,5E+11			3,18E+11	3,46E+41
Combined LR (product)		3,58E+06	5,19E+12	9,53E+09	1,77E+06	5,33E+10	1,88E+09		8,10E+06	4,54E+09	2,26E+14	6,83E+09	0,00E+00		2,14E+10	0,00E+00	2,56E+09	4,04E+09	6,55E+10	0,00E+00	3,52E+11	1,35E+11	2,37E+20
	Number of markers -->	16	27	24	15	23	21		15	21	33	22	0		21	0	22	21	24	0	22	24	34
D3S1358	2,43	2,29	2,44	2,32	2,78	2,88	2,33		2,30	2,30	2,76	2,38		2,38	2,50		2,38	2,25	2,31			2,34	2,48
D19S433	3,18	3,59	2,71	2,55	3,13	2,88	2,33			3,80	3,88	3,54		3,54	3,08		2,55	3,08	3,73		3,08	3,28	2,47
D2S1338	2,35	2,47	3,26	2,99	2,27	1,77	2,94			2,50	4,86	2,38		2,37	2,25		2,35	2,24	2,66		2,25	3,65	2,20
D22S1045	0,72	0,64	0,72	0,72		0,80	0,76			0,70	0,86	0,65		0,65	0,81		0,82	0,75	0,67		0,81	0,74	0,72
D16S539	9,70	9,02	9,97	7,74	5,83	8,86	9,85		7,80	11,10	9,46	10,30		10,30	11,31		8,96	9,82	12,02		11,30	6,88	9,48
D18S51	2,03	2,00	1,62	1,60	2,08	1,81	2,00		2,20	1,80	1,88	2,03		2,03	2,03		2,19	1,80	1,81		2,03	2,32	2,46
D1S1656	4,73	4,89	4,26	3,80		6,60	6,38			3,40	4,73	5,01		5,01	6,93		3,43	4,60	4,65		6,94	6,27	7,00
D10S1248	8,45	8,22	9,09	6,88		7,02	7,23			11,10	8,68	9,03		9,03	8,92		6,68	6,24	12,24		8,93	7,54	120,25

Wet exercise

➢ A single alleged father

Note: log scale on y-axis
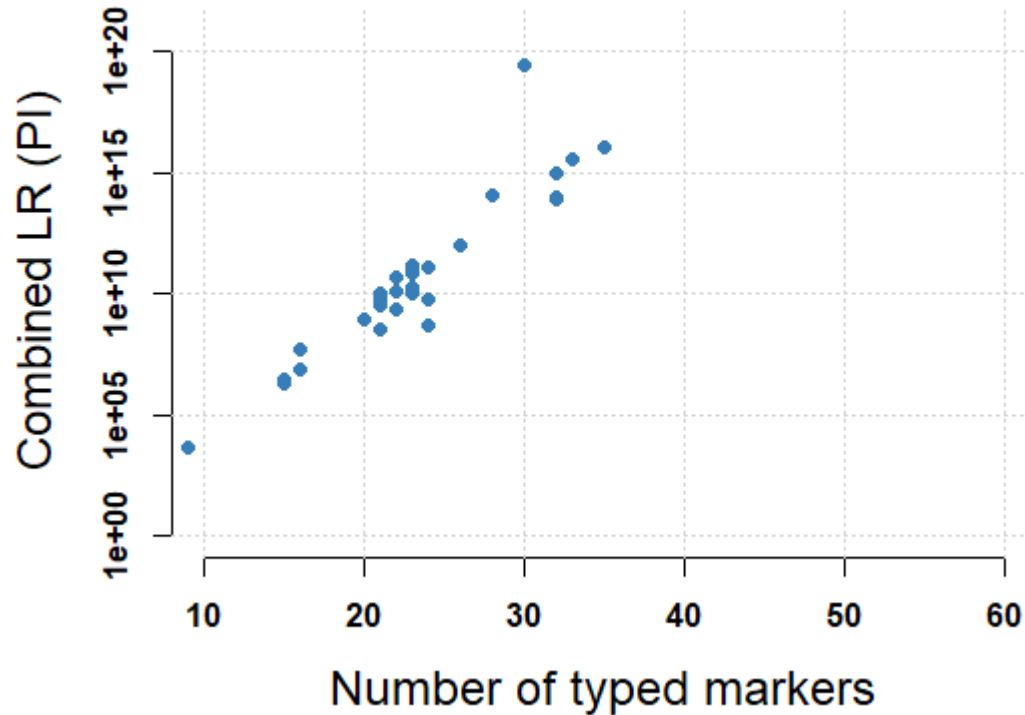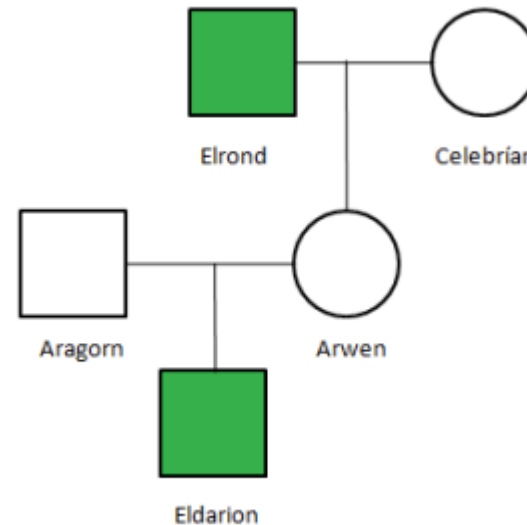
Wet exercise – per marker LR variation

Results for the most commonly typed STRs
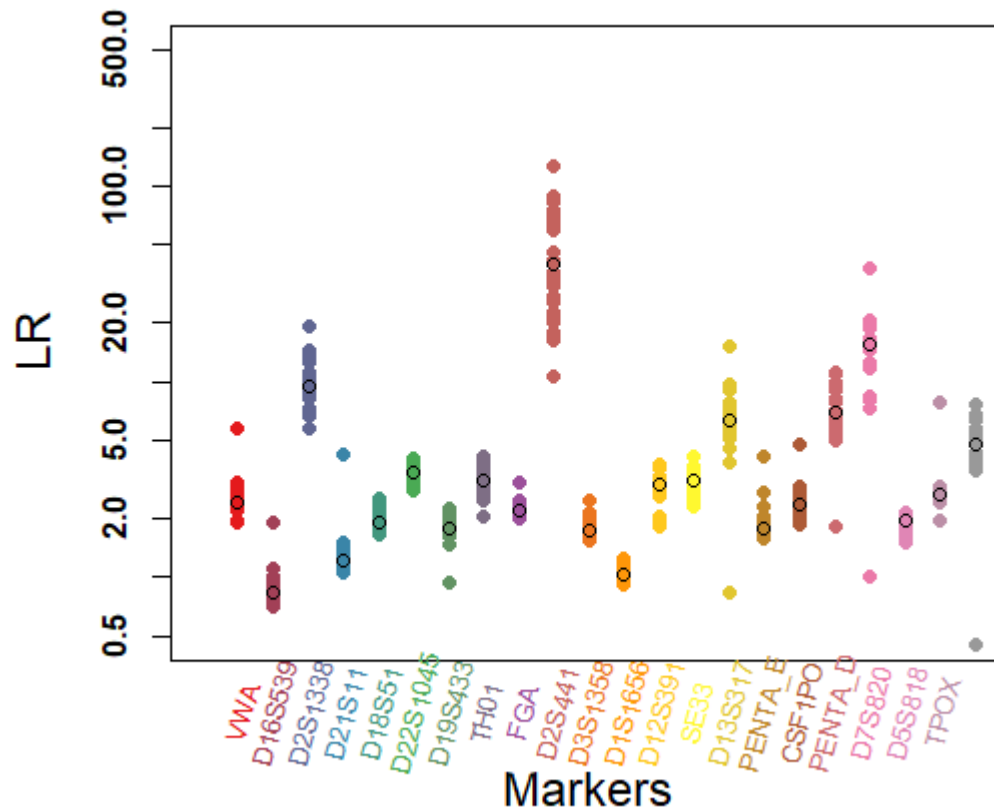
PAPER CHALLENGE

Paper challenge - Background

- Grandparent/grandchild case
- Autosomal data
- Y-data

Paper challenge – Setup

ESWG PAPER CHALLENGE 2024

This year's paper challenge consists of a single exercise divided into three parts. In order to obtain the certificate, participants have to submit results for part a), whereas part b-c are optional. All data is given as files at https://familias.name/ESWG/ESWG2024_paperchallenge.zip in addition to some details given directly in the cases. Please fill out all answers in the supplied Excel questionnaire.

The legacy of elves and man

History has it that in a hidden valley where the air was fragrant with the whispers of ancient trees, Elrond Half-elven ruled as a wise and compassionate lord. His lineage was unique: part mortal, part immortal. But it was Aragorn, the heir of Isildur, who would forever alter the course of their lives. Aragorn, raised by Elrond as a foster son, had grown into a formidable warrior. His destiny lay in reclaiming the throne of Gondor, yet his heart yearned for Arwen Undómiel, Elrond's beloved daughter. Arwen, radiant as moonlight on a tranquil lake, had captured Aragorn's soul from the moment they met. Their love was forbidden, for Elrond foresaw the pain it would bring. He knew the choice that awaited Arwen: to remain immortal or to bind her fate to Aragorn's mortal life. Ultimately the union of Arwen and Aragorn was completed when their son, Eldarion was born.

The DNA mystery began when Elrond, in his vast library, discovered an ancient scroll. Its faded ink revealed a forgotten prophecy: "When the blood of Elves and Men mingles, a hidden power shall awaken." Elrond sensed that this prophecy held the key to their intertwined destinies and in particular for Eldarion. However, before Elrond could untangle the meaning of the prophecy he was mortally wounded.

In another age, called the modern age by some, archaeologists unearth graves bearing the inscriptions Elrond and Eldarion. We are baffled by the possibility that this could indeed be the remains of the historical persons alluded to above.

Paper challenge – Part a)

a) Based on the STR DNA data given in Table 1, compute the likelihood ratio comparing the hypothes

       H1: Elrond is the maternal grandfather of Eldarion

       H2: Elrond is unrelated to Eldarion
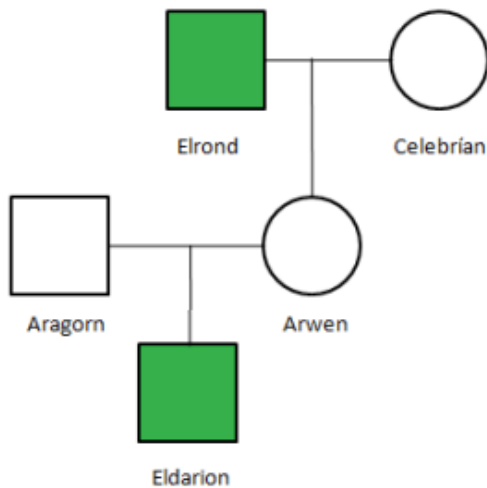
Paper challenge – Part a)

➢ LR=101.83 (Reported by almost all labs)

➢ Some labs accounted for linkage and got a slightly different LR

Paper challenge – Part b)

b) Another excavation uncovers a third grave bearing the inscription *Galadriel*. From history books you learn that this could be the legendary *Lady of the Golden Wood*. From the old records you find the pedigree depicted below. Based on the DNA data in Table 2, find if Galadriel, Elrond and Eldarion are related as depicted in Figure 2. Construct relevant pedigrees for the comparison and report LR and posterior probabilities.
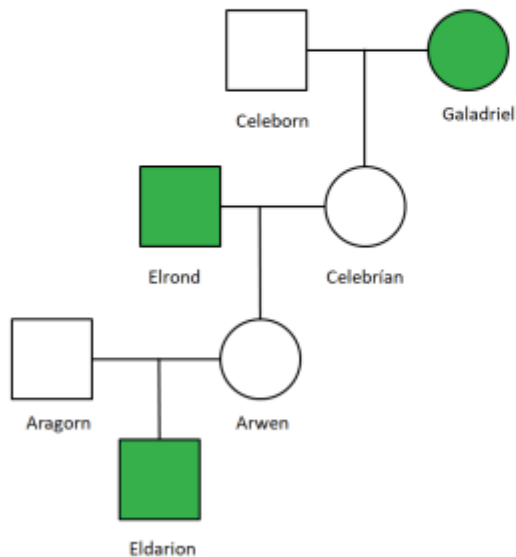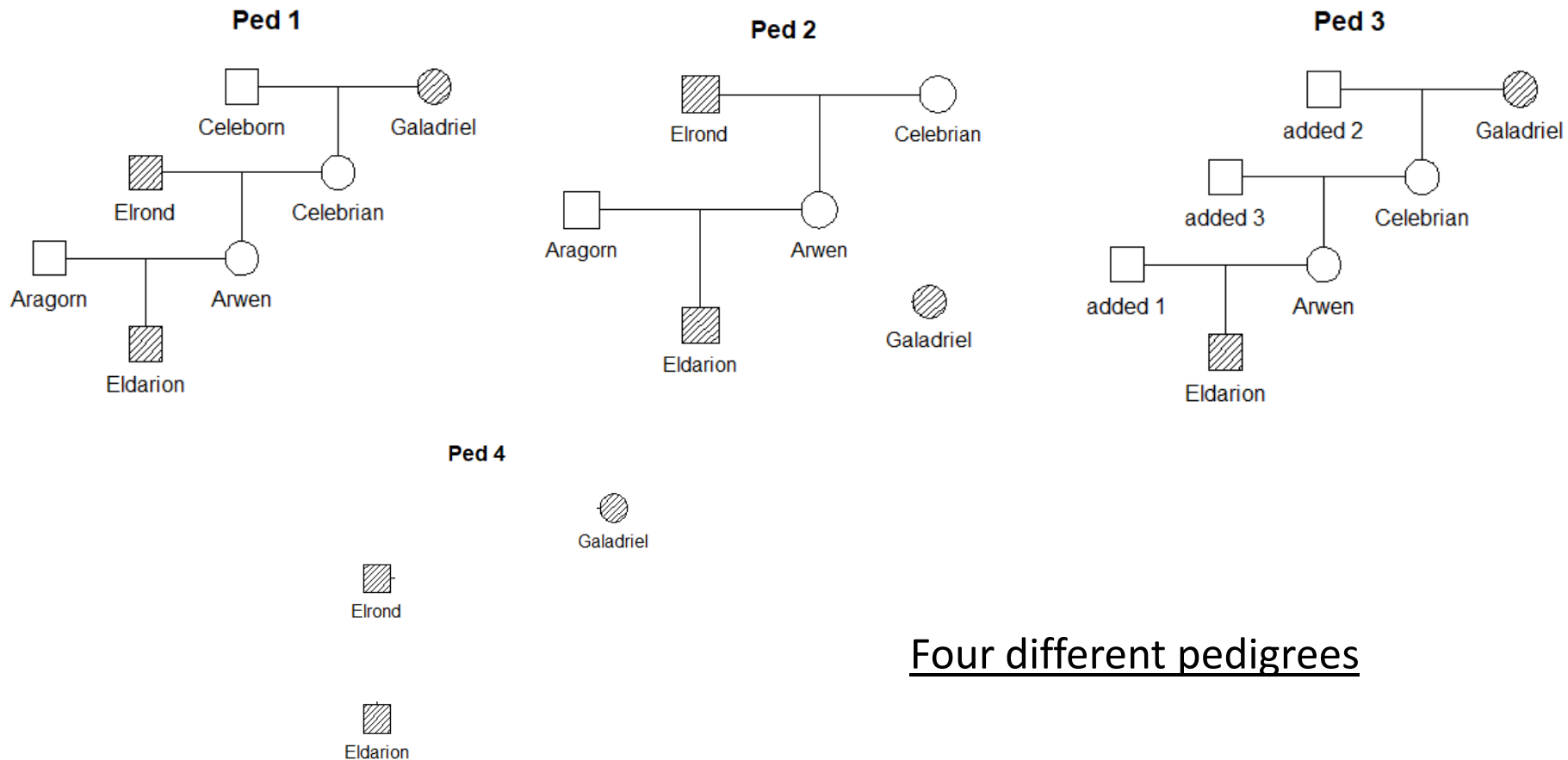

Three typed individuals
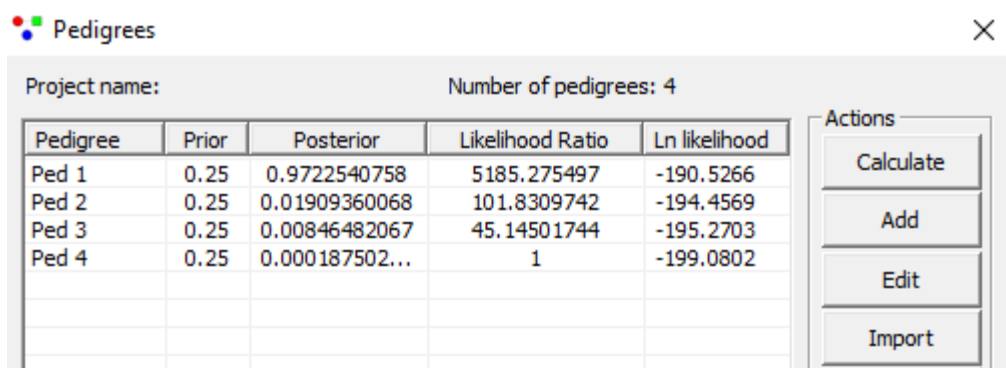Challenge: Construct relevant pedigrees

Demonstration in Familias

Paper challenge – Part b)

Four different pedigrees

Paper challenge – Part b)

Pedigree	Prior	Posterior	Likelihood Ratio	Ln likelihood
Ped 1	0.25	0.9722540758	5185.275497	-190.5266
Ped 2	0.25	0.01909360068	101.8309742	-194.4569
Ped 3	0.25	0.00846482067	45.14501744	-195.2703
Ped 4	0.25	0.000187502...	1	-199.0802

Some different approaches

   i.   Pairwise (three pedigrees vs Ped 4)

  ii.   Joint (all four pedigrees)

 iii.   Only Ped 1 vs Ped 4 (or Ped 1 vs Ped 2)

Paper challenge – Part c)

c) It is told that Aragon (see Figure 3 below) is connected through a long unbroken paternal lineage to Elrond. Running a Y-STR analysis you are able to obtain profiles for comparison. We can assume Elrond and Aragorn are separated by 65 generations. In the calculations you can assume that the haplotype of Elrond is the founder haplotype and has been observed 10 times while the haplotype of Eldarion has never been observed. The size of the Y-database is 289,406. For simplicity we can assume that the mutation rate is equal to 0.001 for all included markers and that there is an equal chance for a loss or gain of a tandem repeat in the mutation model. Further assume that there is a 90% chance for a mutation to be single step, 9% two step and so forth.
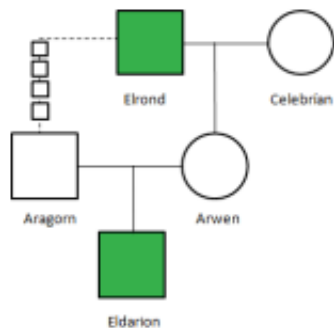
Challenge: A large number of generations!

Paper challenge – Part c)

Table 3. Marker data for the two individuals involved in part c).

Marker	Elrond	Eldarion
DYS19	14	14
DYS389I	13	13
DYS389II	29	30
DYS390	24	24
DYS391	10	10
DYS392	11	11
DYS393	12	12
DYS385	14,17	14,17
DYS437	15	15
DYS438	10	10
DYS439	12	12
DYS448	21	21
DYS456	15	15
DYS458	16	16
DYS635	21	21
YGATAH4	10	10
DYS481	22	22
DYS533	11	11
DYS549	12	12
DYS570	17	17
DYS576	15	15
DYS643	11	11

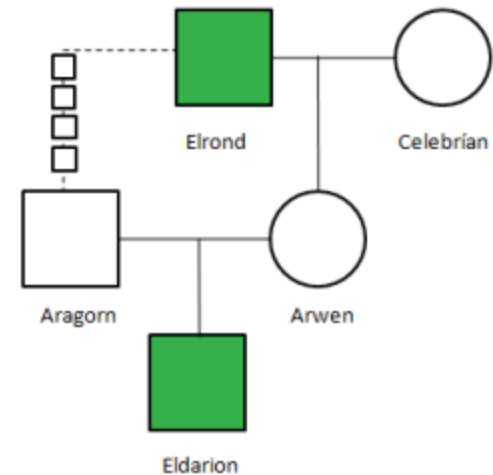One mutation!

A variety of LRs presented!

Oslo
University Hospital

Demonstration YHRD

Paper challenge – Part c)

> Manual derivations
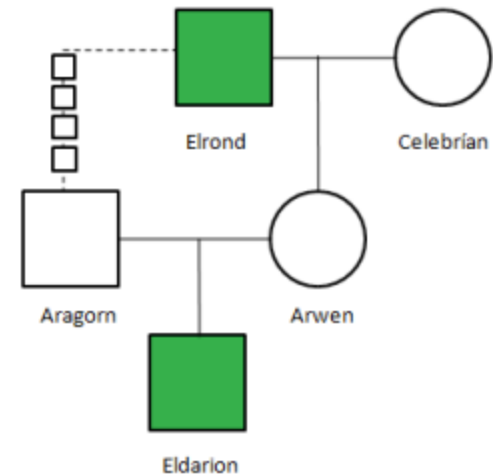
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b) One marker (DYS389II) with possible mutation
c) 22 STR markers without mutations (DYS385 counted twice)
d) Mutation rate (mu) = 0.001
e) No mutation = (1-mu) = 0.999
f) Mutation increase = mu/2 = 0.005
g) Single step increase = mu/2 * 0.9 = 0.0045
h) One single step increase mutation in any generation = (mu/2 * 0.9)*66*(1-mu)^65=0.0278
i) No mutation single marker = (1-mu)^66 = 0.936
j) No mutation 22 markers = ((1-mu)^66)^22 = 0.23

Elrond   Celebrían

Aragorn   Arwen

Eldarion

Paper challenge – Part c)

➤ Manual derivations

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H1: Elrond is the paternal ancestor of Eldarion
H2: Elrond and Eldarion are unrelated

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Paper challenge – Part c)

Elrond Celebrían

Aragorn Arwen

Eldarion
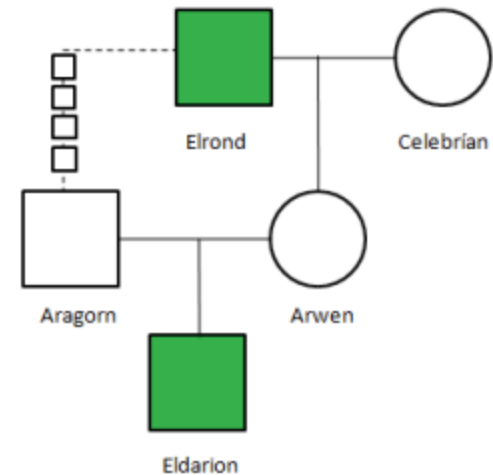
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L (H2) = H_Elr * H_Eld

Paper challenge – Part c)

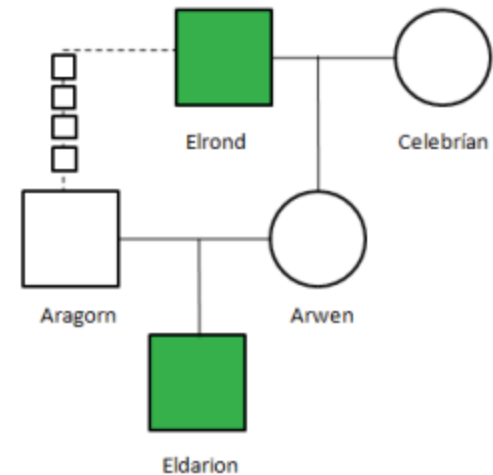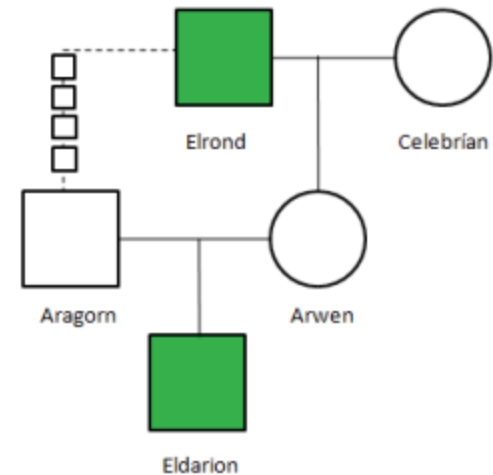> Manual derivations

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L (H1) = H_Elr * [no mutations in 22 markers over 66 generations…
a single mutation in 1 marker over 66 generations..]

Paper challenge – Part c)

> Manual derivations

Elrond Celebrían

Aragorn Arwen

Eldarion

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L (H1) = H_Elr * [(1-mu)^66]^22 * (mu/2 * 0.9) * 66 * (1-mu)^65

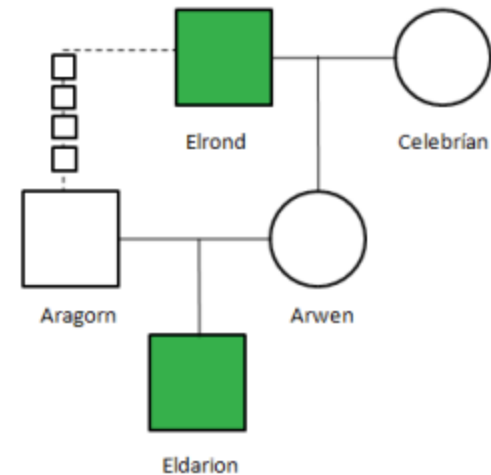# Paper challenge – Part c)

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H1: Elrond is the paternal ancestor of Eldarion
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LR = L(H1)/L(H2) = [H_Elr*[(1-mu)^66]^22*(mu/2 * 0.9)*66*(1-mu)^65]/[H_Elr*H_Eld]
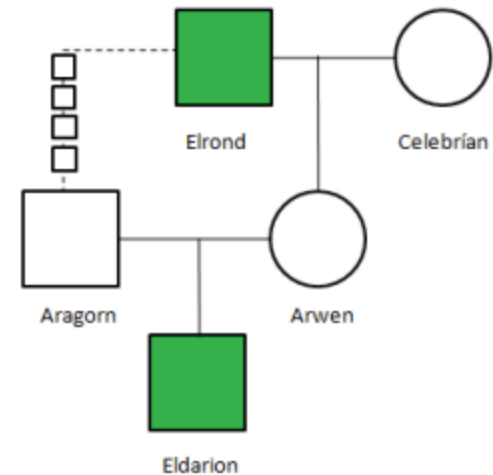
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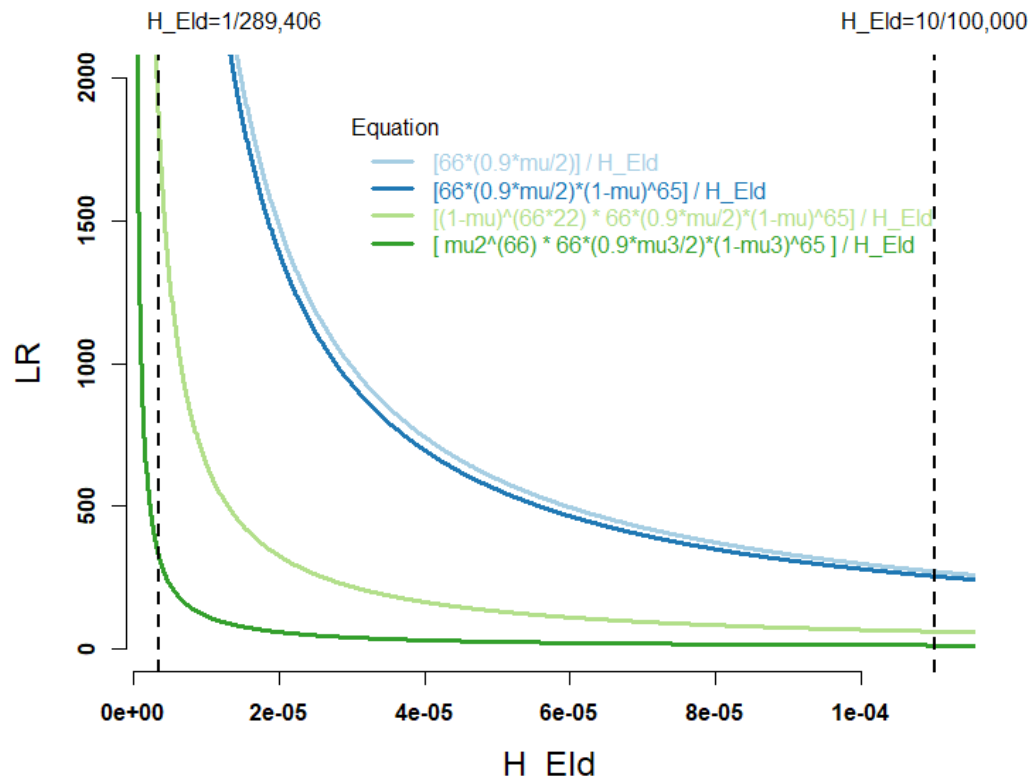LR = L(H1)/L(H2) = [0.23*0.0278]/H_Eld

Paper challenge – Part c)

➢ Results

Where mu=0.001, mu2=Product of YHRD (1-mutation rates) and mu3 is the mutation rate for DYS389II

Paper challenge – Part c)

➢ Reported LR

1. YHRD -> LR=131 (with Worldwide population haplotypes and H_Eld=1 / 103,280), which can be adjusted for the size given in the exercise such that the LR= (LR/103,280)*289,406 = 367

2. Manual formulas -> Ranging from 100 to 10000, as is expected for Y where YHRD use locus-specific mutation rates.

Paper challenge – Summary

➢ Video will be available through https://familias.name/ESWG/

➢ SNP test presented separately

ESWG - Proficiency test 2024

Daniel Kling – Head Organizer

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