GBSathon

Benchmarking reproducibility of Genotyping-By-Sequencing analysis workflows through comparison with SNP chip and pedigree data

Rachael Ashby, Rudiger Brauning, Hayley Baird, Ruy Jauregui, Monica Vallender, Aurelie Laugraud, Charles Hefer, Abdul Baten, Paul Maclean, Rayna Anderson, Roger Moraga, Siva Ganesh, Tracey van Stijn, Jeanne Jacobs, Ken Dodds, John McEwan, Shannon Clarke and Andrew Griffiths

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Apologies

- Rachael can’t be joining us this week.

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- Crown Research Institute
- Delivering cost effective, high-quality, high-throughput solutions to primary industry and more

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United in Data, but…

- when working with GBS data we noticed that people have different favourite tools

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The challenge

- Find the best GBS analysis workflow
- Convince your peers

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The team

- Lab
  - Hayley Baird, Rayna Anderson, Tracey van Stijn
- Bioinformatics
  - Rachael Ashby, Rudiger Brauning, Ruy Jauregui, Monica Vallender, Aurelie Laugraud, Charles Hefer, Abdul Baten,, Roger Moraga
- Stats
  - Paul Maclean, Siva Ganesh, Ken Dodds
- PI
  - Jeanne Jacobs, John McEwan, Shannon Clarke, Andrew Griffiths

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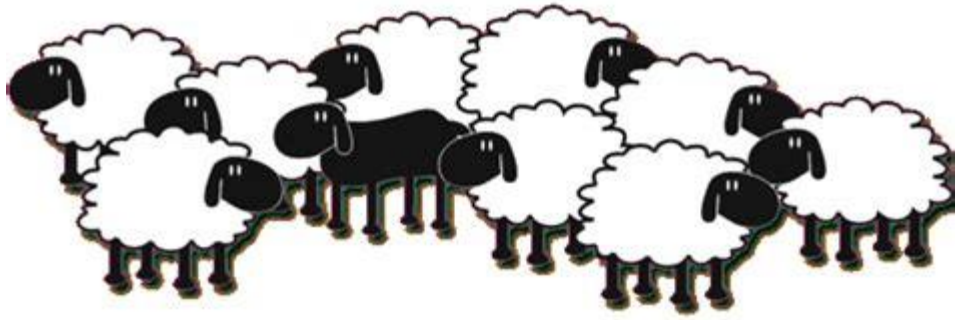
The test species



- Sheep, well behaved diploid species 3Gbp genome
- Previously pooled samples of highly heterozygous species that shall remain unnamed

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Hold on a minute, what's GBS?

- RRS 1-10% of the genome depending on RE (PstI-MspI)
- Multiplexed samples
- Genotypes
- Like a custom SNP chip, fitting your samples perfectly
- Range of downstream applications (breeding values, parentage test, population structure, …)

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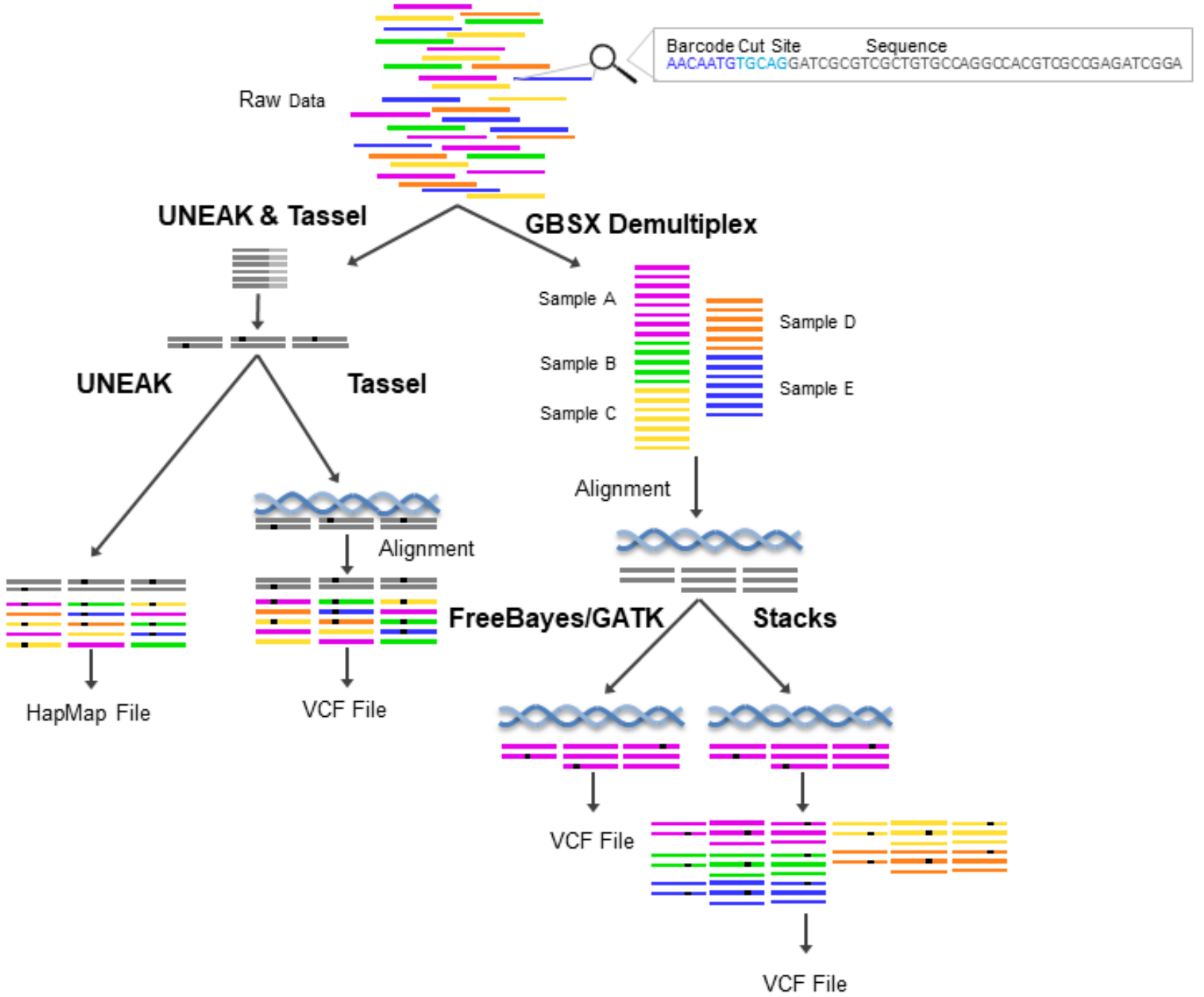
Pipelines

- Different pipelines, with/without reference, NR/full depth, full length/64bp, 1 or more SNPs per tag, WGS vs RRS, Bayesian (prior knowledge) or not



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Tools, an acquired taste

- Funny requirements like restrictions around chromosome names
- User-friendly?
- Compute expense (time, threads, RAM)
- Conda packages

pipeline	max RAM [GB]	compute time [dd]
bwa	9	10.20
GBSX	1	1.50
freebayes	35	4.00
GATK	14	23.00
Tassel5	49	0.04
Tassel3	10	0.04
Stacks	25	0.80
Samtools	2	2.00
UNEAK	26	0.17

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Tools, some surprises…

- ‘unsupported’ GT
- Indels
- N SNPs
- duplicate positions, …

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How to compare?

	SNP-calling Pipeline						
Statistics	STACKS	GATK	Freebayes	SM	Tassel3	Tassel5	UNEAK
Number of SNPs	210 044	338 692	188 131	461 506	264 136	210 779	111 053
Call rate	0.58	0.69	0.89	0.58	0.91	0.80	0.69
Mean sample depth	5.18	5.26	10.78	4.46	13.29	8.74	6.30
Mean co-call rate (sample pairs)	0.44	0.59	0.83	0.49	0.86	0.71	0.59
Mean Inbreeding	0.26	0.31	0.10	0.09	0.21	0.12	0.08

- Gold standard (SNP chip) & biology (pedigree)
- End results (GRM)

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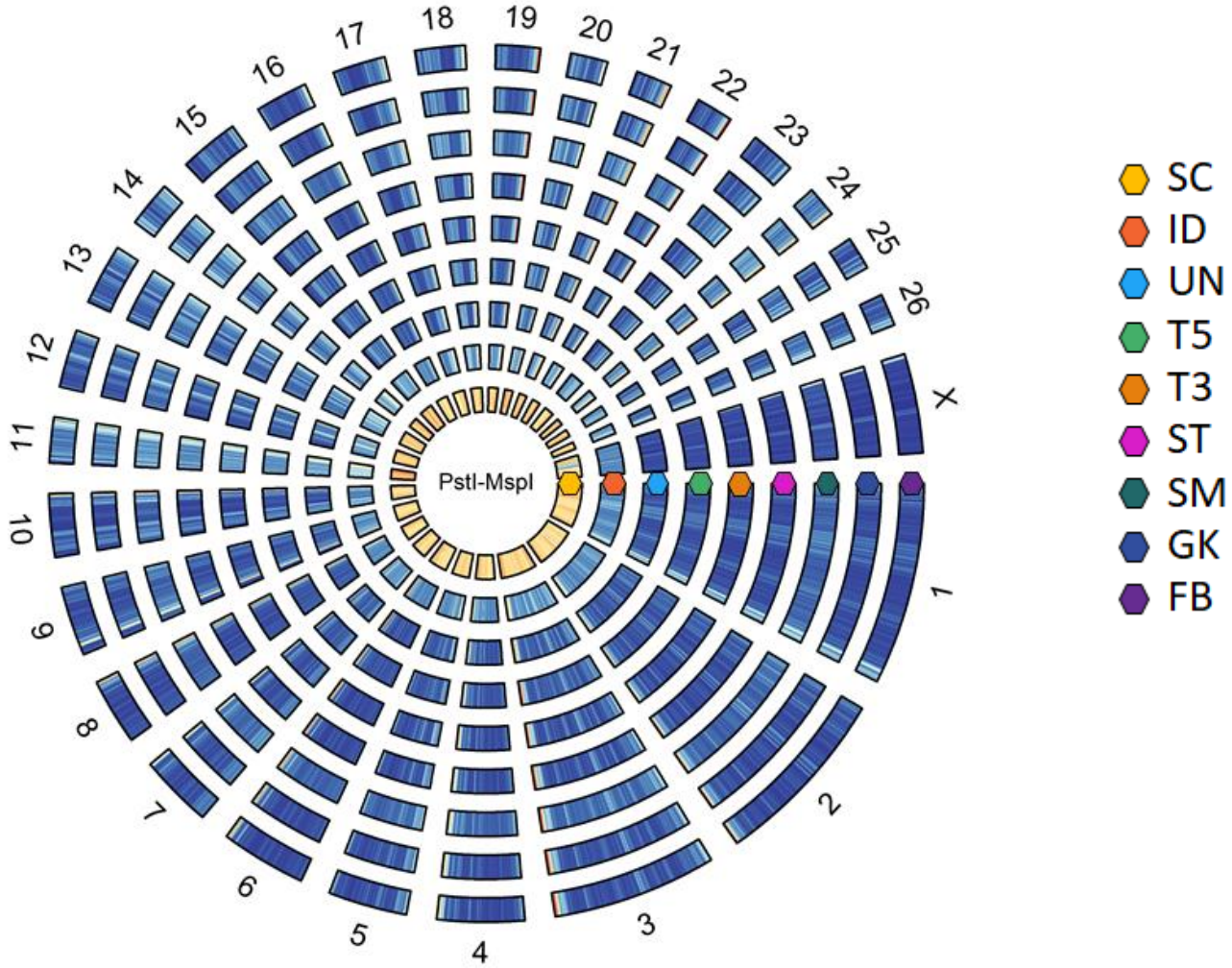
Relative genomic coverage



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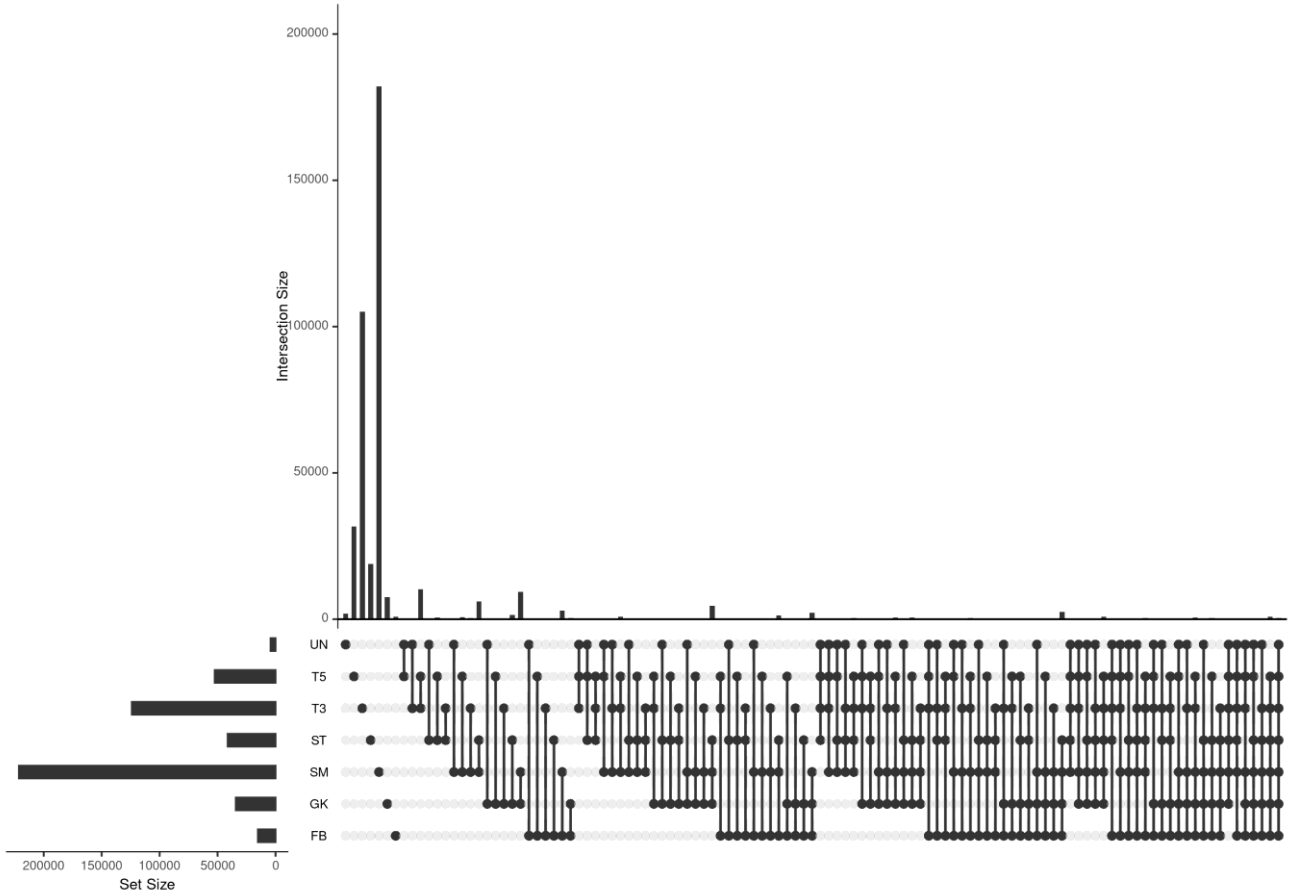
SNP sets

- Upset plots, dbSNP



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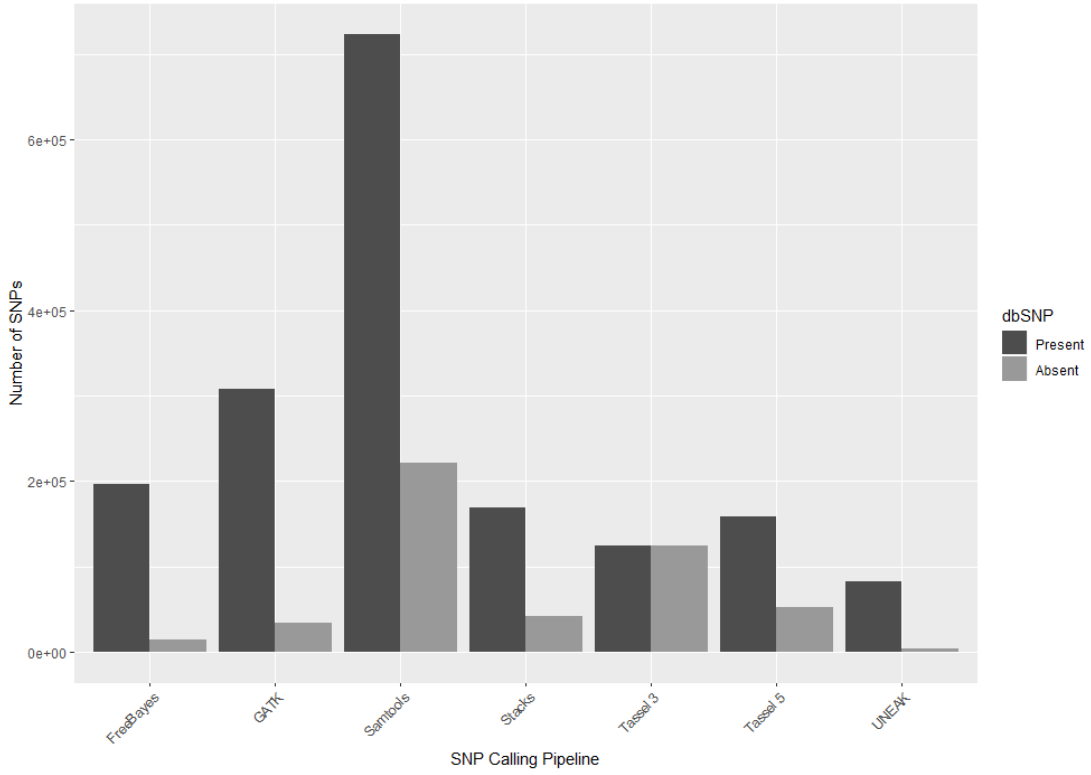
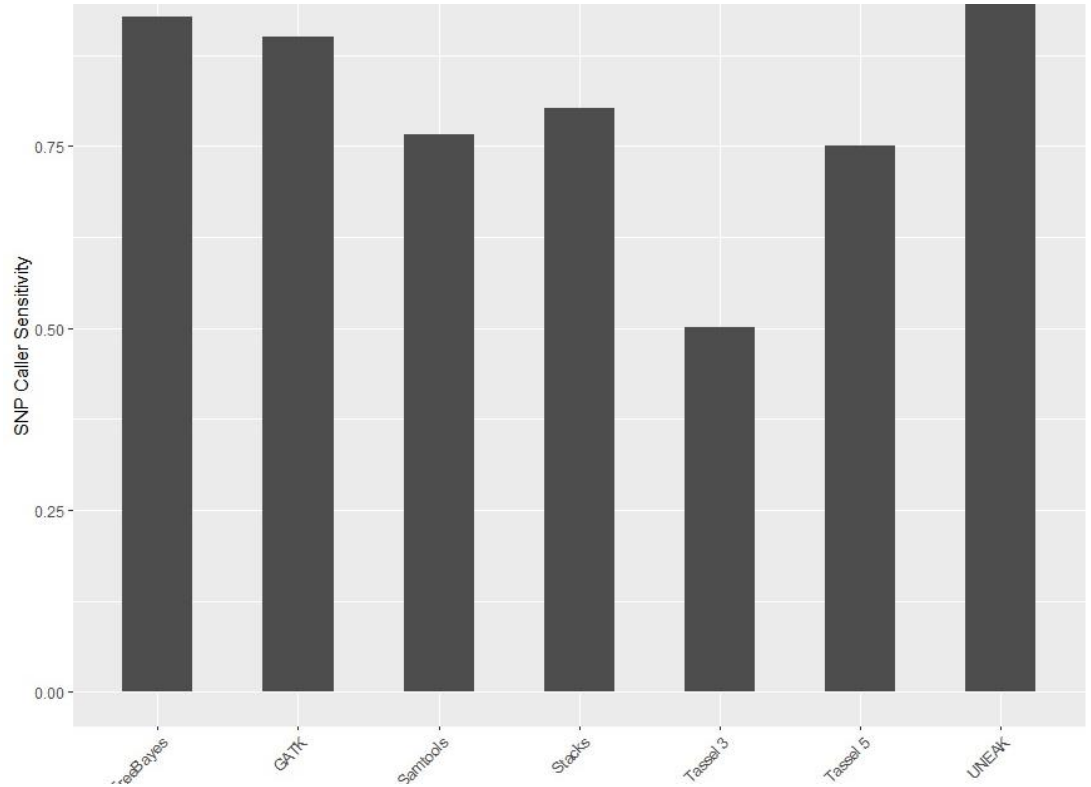
SNP sets vs dbSNP





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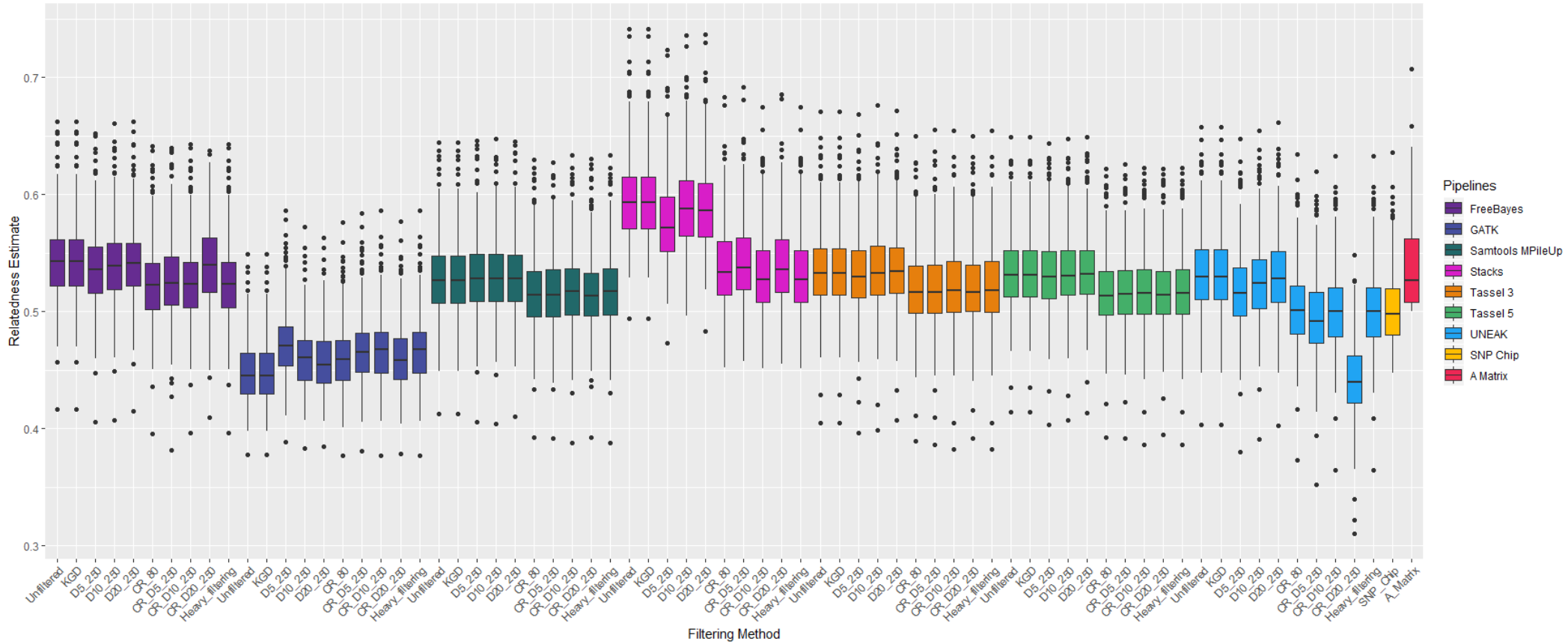
Relatedness, dams, etc.



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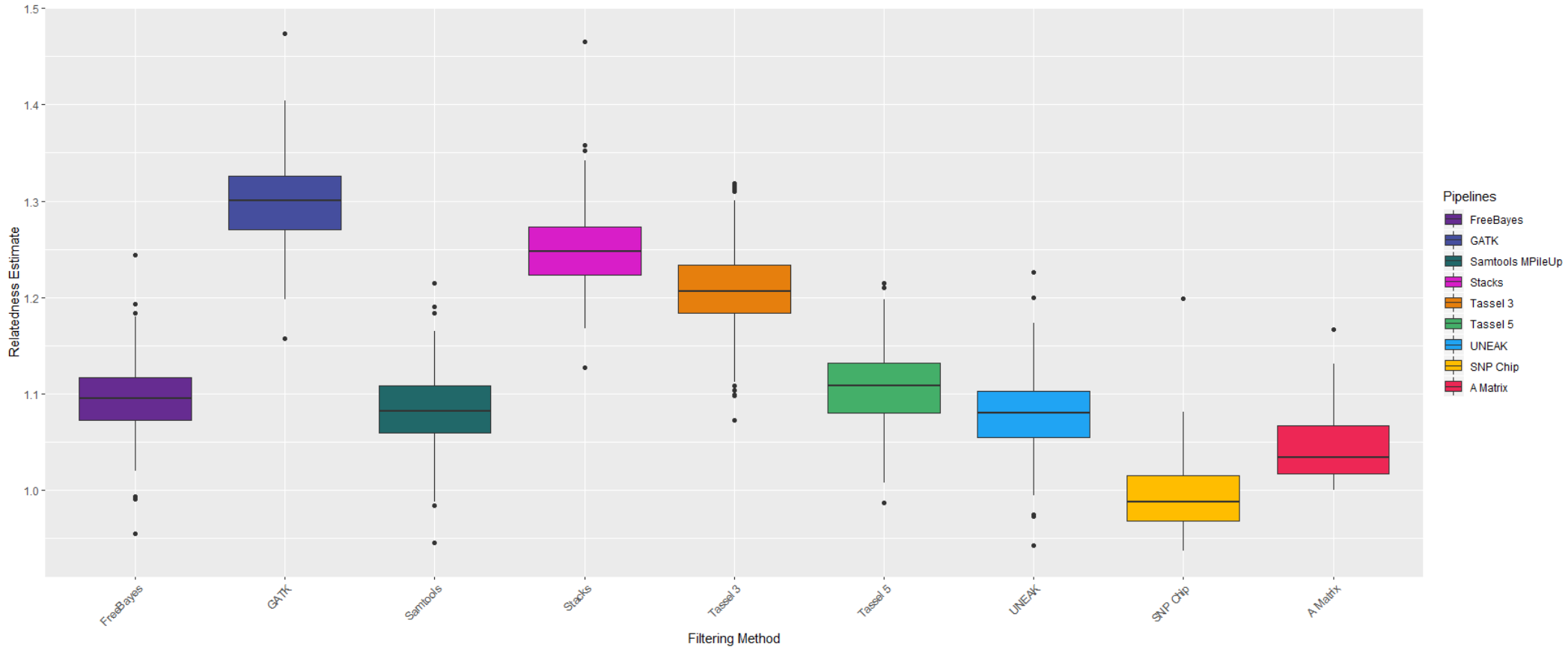
Inbreeding



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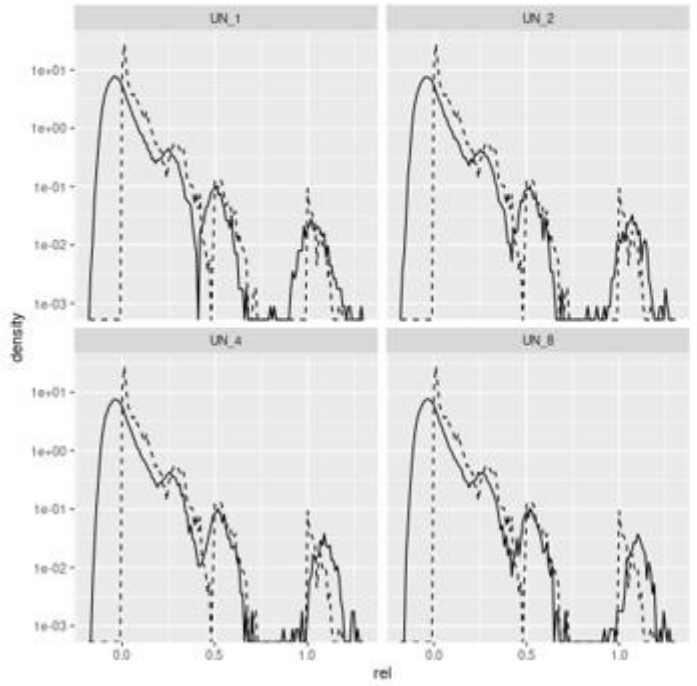
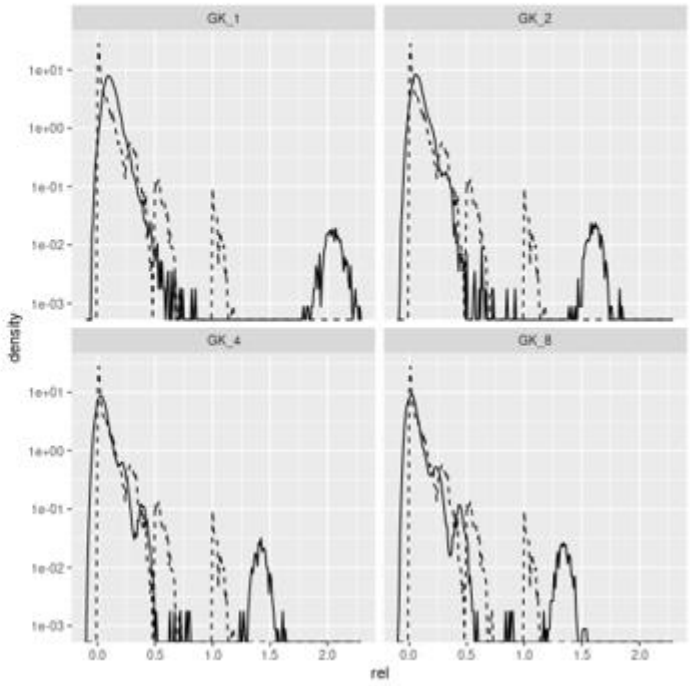
Relatedness matrix, plotted

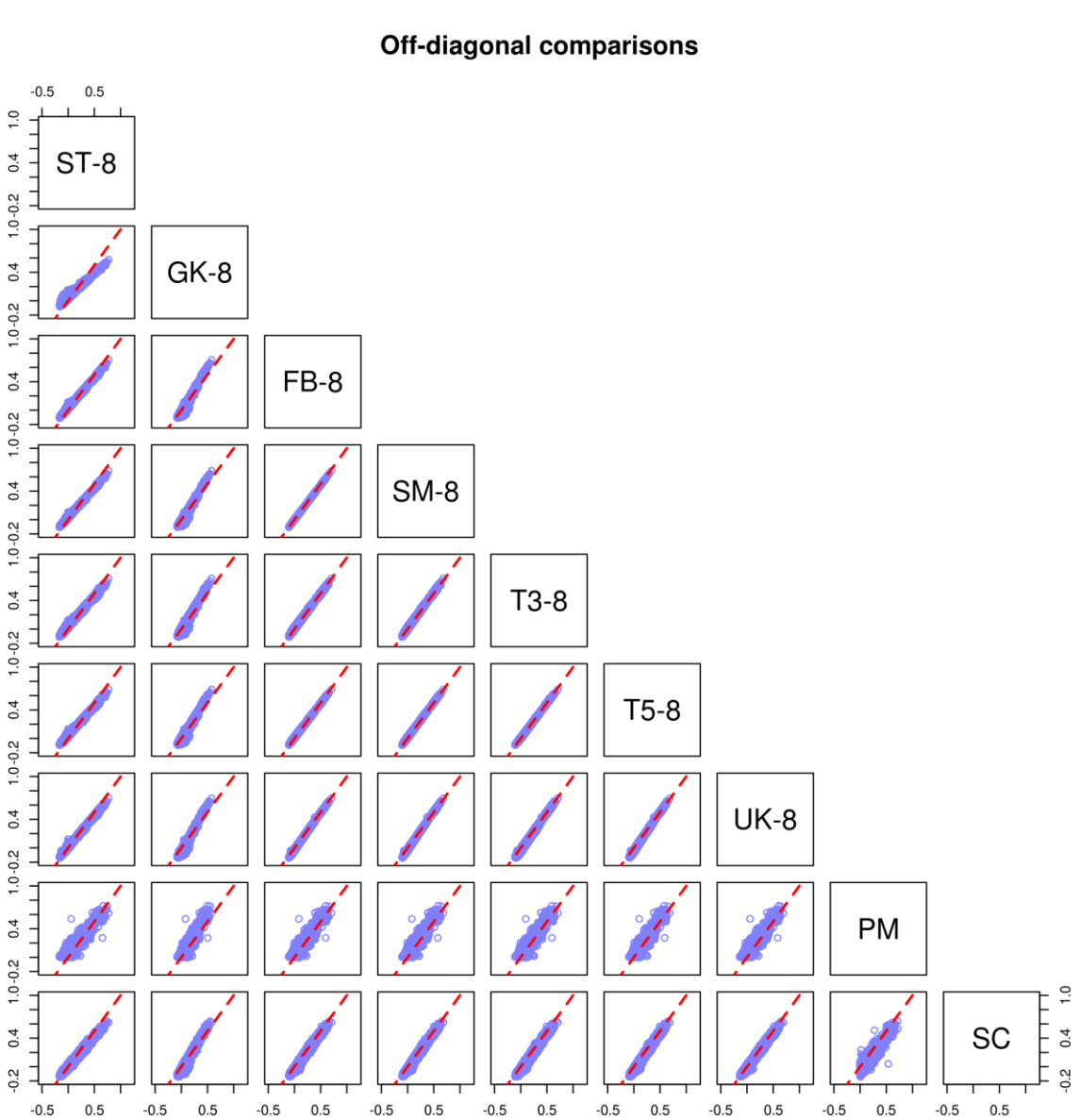
UN

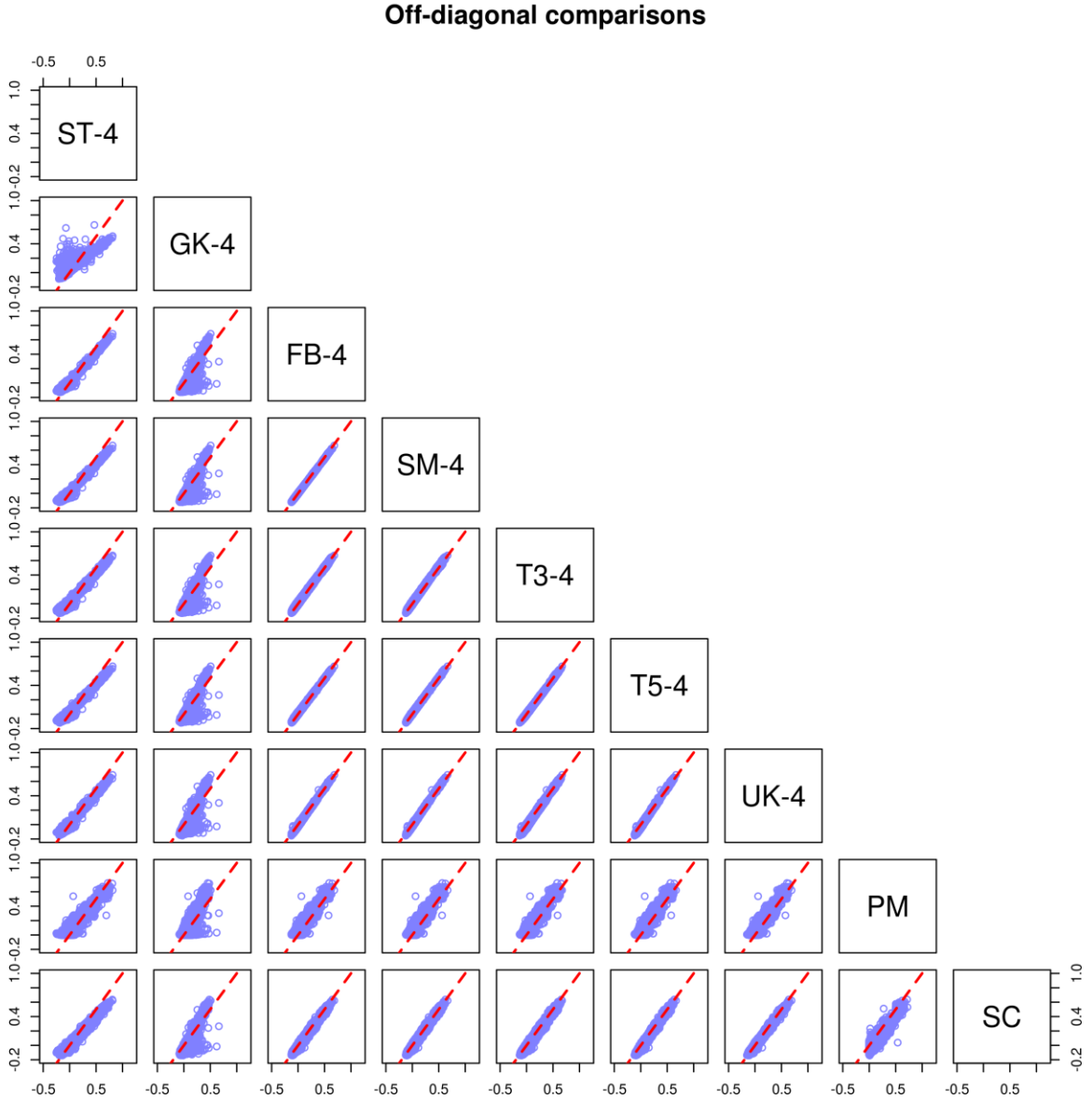


GK



Lowering the depth





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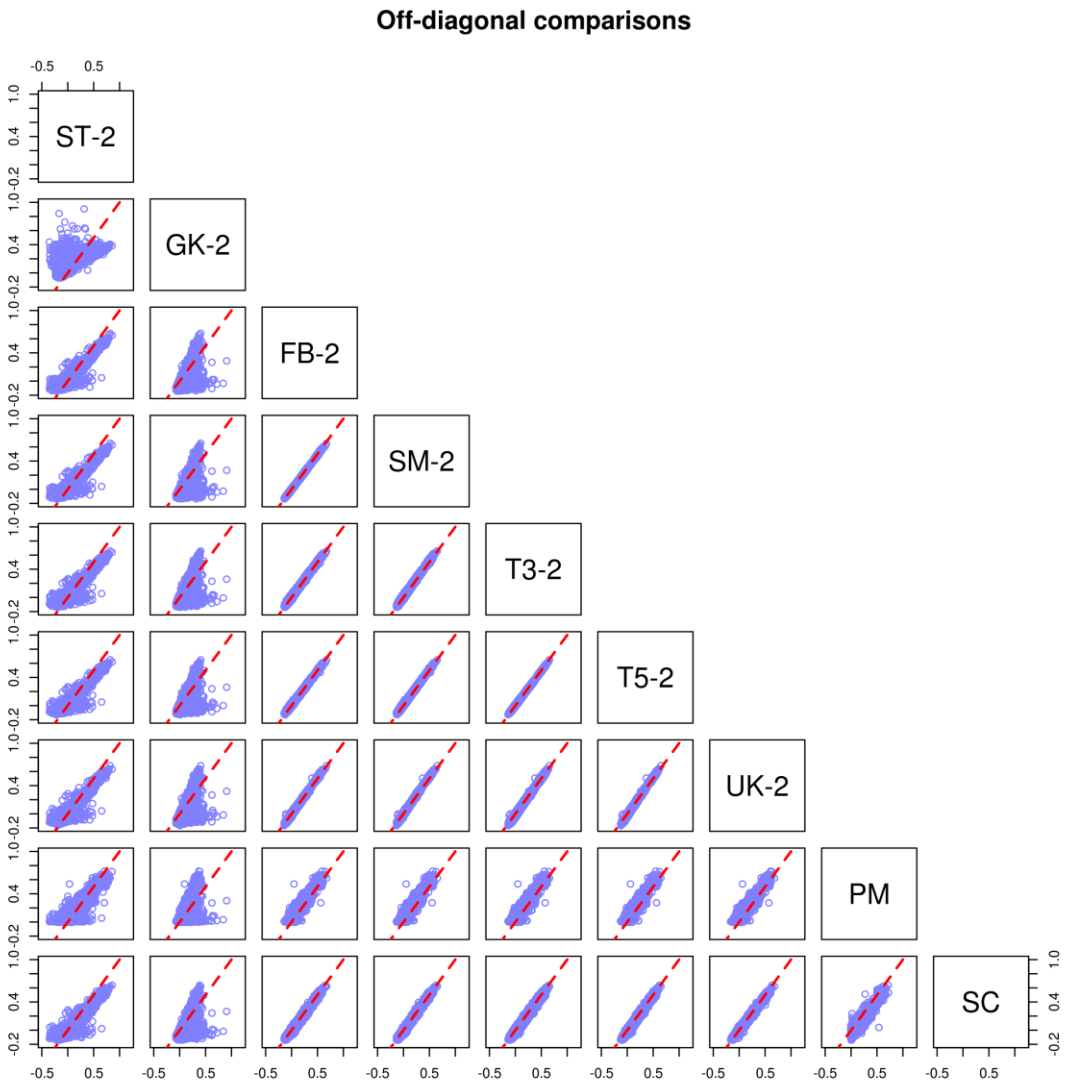

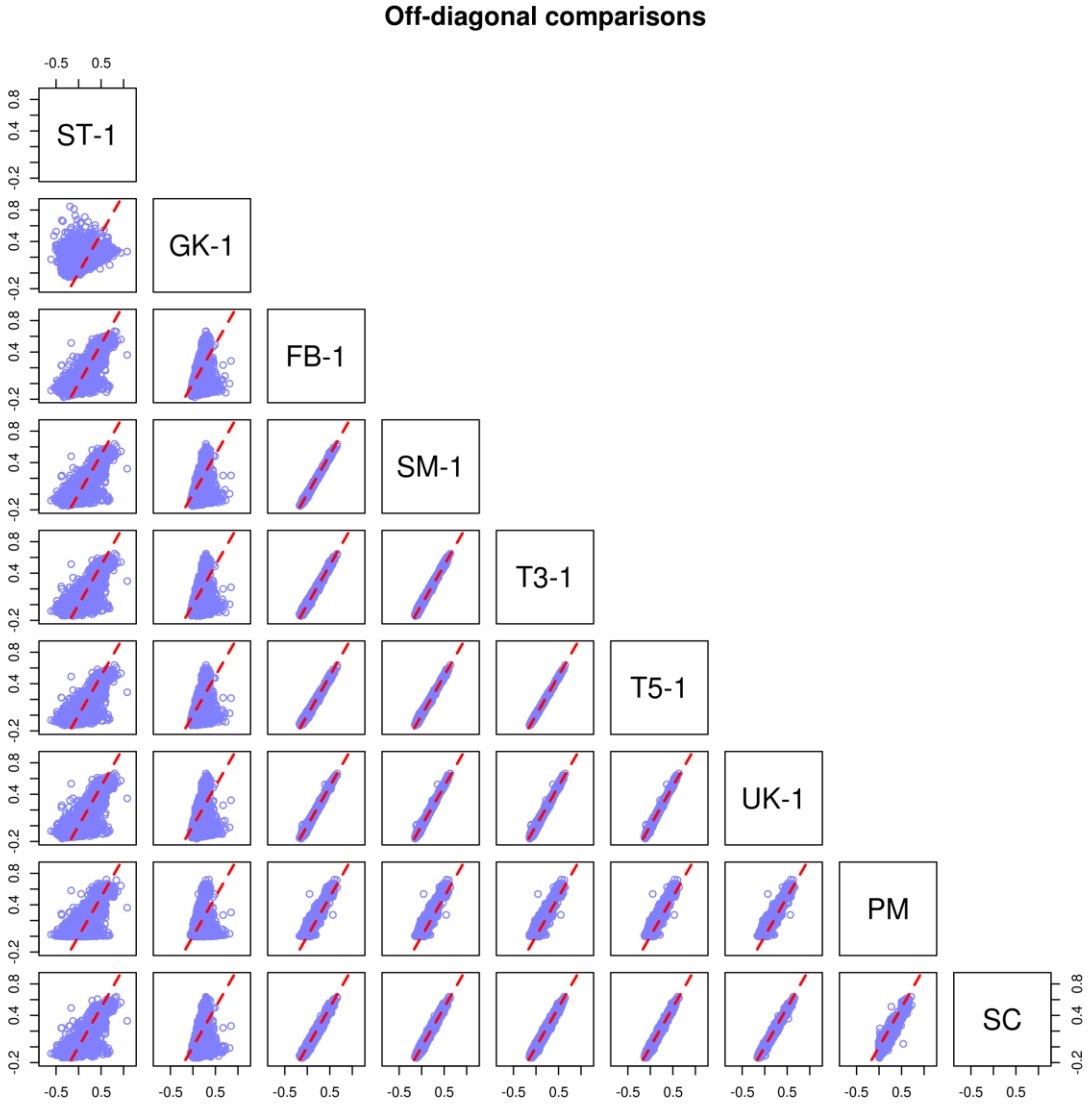

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Low depth can work!

- Our tricks:
- problematic absolute GT, therefore use RA to get probabilistic genotypes
- don’t require callrate of .8, instead create relationship matrix stepwise by doing all possible pairwise comparisons (more SNPs can be utilized)
- HW filter to get rid of repetitive sequence
- Finplot

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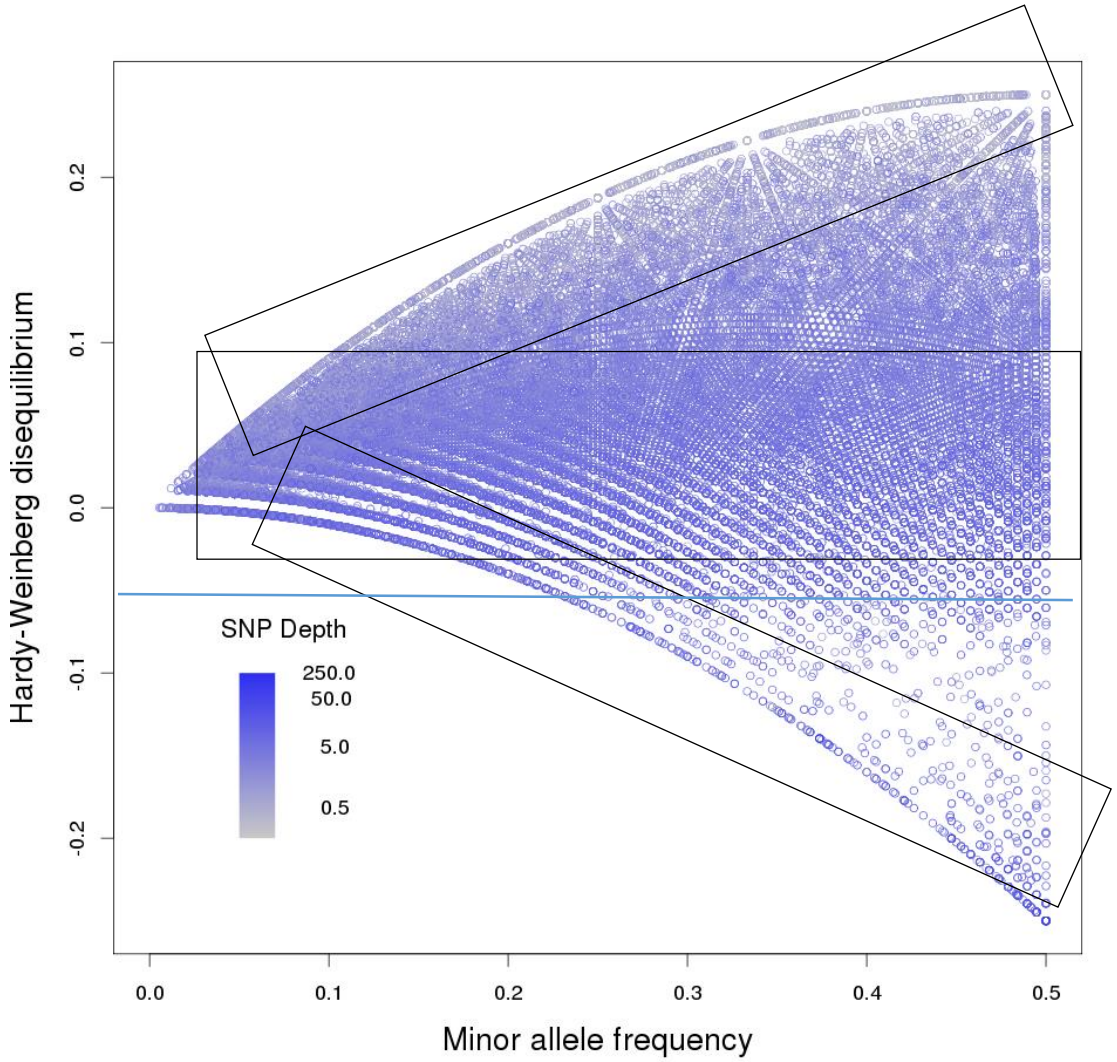
Sheep example



Low depth
appear homozygous

Intermediate depth
in HWE

remove SNPs below line

High depth
maximum heterozygosity
Possibly duplication regions

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Lessons learned

- naming convention
- version control
- thoroughly check results before moving on
- gold standard
- biology

- Thanks!
- More on biorxiv soon….