Missing ROH ?

Recommendations for tuning PLINK in runs of homozygosity analyses on medium density genotypes

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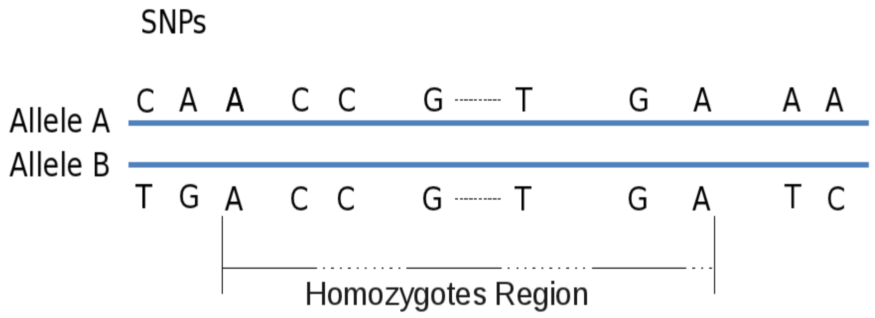
Runs Of Homozygosity

- Homozygous segments assumed to arise from a common ancestor



- State-of-the-art method for inbreeding analyses

- Detection of ROH islands as signatures of selection

→ PLINK (v1.9, Chang et al. 2015) is commonly used for ROH detection

PLINK ROH detection algorithm

Scanning window approach:

1. Scanning window definition

   (-window-snp, -window-missing and -window-het)

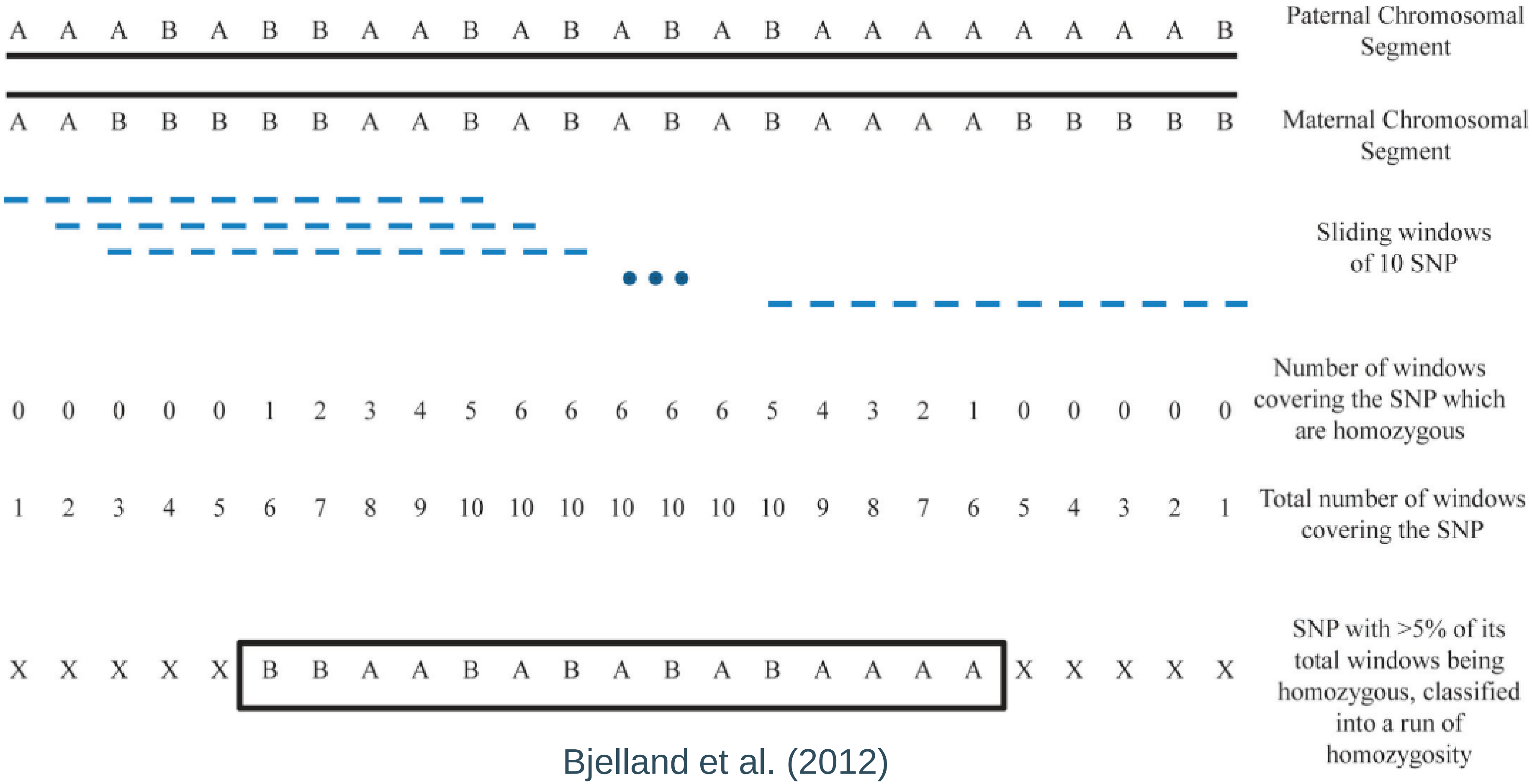
2. Every individual SNP's proportion of appearance in homozygous windows is calculated

3. SNPs passing threshold → potential ROH

4. Extra constraints to identify ROH

   (-gap, -density, -snp, -kb and -het)

PLINK ROH detection algorithm



A	A	A	B	A	B	B	A	A	B	A	B	A	B	A	B	A	A	A	A	A	A	A	A	B	Paternal Chromosomal Segment
A	A	B	B	B	B	B	A	A	B	A	B	A	B	A	B	A	A	A	A	B	B	B	B	B	Maternal Chromosomal Segment
																									Sliding windows of 10 SNP
0	0	0	0	0	1	2	3	4	5	6	6	6	6	6	5	4	3	2	1	0	0	0	0	0	Number of windows covering the SNP which are homozygous
1	2	3	4	5	6	7	8	9	10	10	10	10	10	10	10	9	8	7	6	5	4	3	2	1	Total number of windows covering the SNP
X	X	X	X	X	B	B	A	A	B	A	B	A	B	A	B	A	A	A	A	X	X	X	X	X	SNP with >5% of its total windows being homozygous, classified into a run of homozygosity

Bjelland et al. (2012)

Impact of (PLINK) settings on ROH detection

Data quality control

➡ Pruning for low MAF

➡ Pruning for LD

PLINK settings

Scanning window

- --homozyg-window-snp
- --homozyg-window-het
- --homozyg-window-missing
- --homozyg-window-threshold

ROH definition

- --homozyg-snp
- --homozyg-kb
- ➡ --homozyg-density
- --homozyg-gap
- --homozyg-het

Material and Methods

- Review of recent papers (pigs, cattle, sheep, horses,…)
- Test all (previously undiscussed) settings independently
- Use own and publicly availabel data of different species and populations

Material and Methods

Basic testing conditions

- No MAF pruning
- No LD pruning
- Density 200 $\frac{kb}{SNP}$
- Gap 2 Mb
- Threshold 0.05
- Scanning window size 20 SNPs
- Minimal ROH length 1 Mb
- Minimal number of SNPs calculated by Purfield et al. 2012
- Maximum 1 missing SNP and no heterozygous SNPs

Genome Coverage

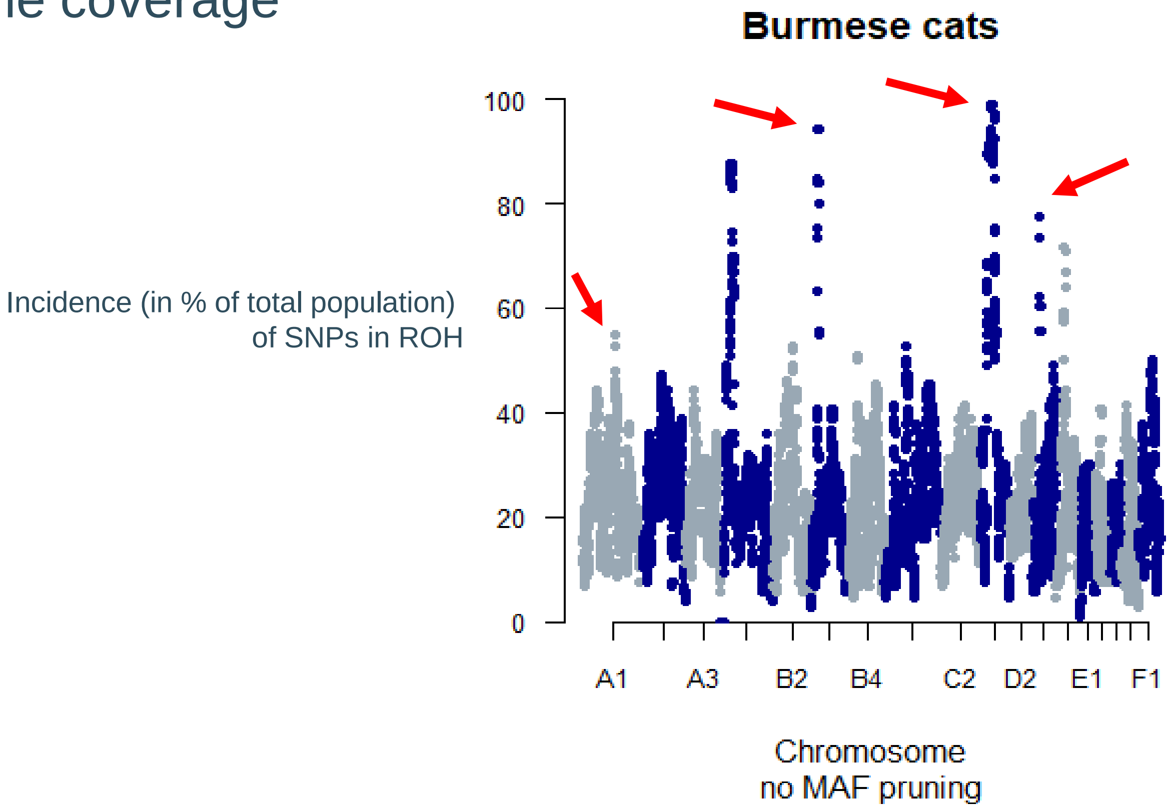
$$Genome\ coverage = \frac{ROH_{max}}{Total\ genome\ length}$$

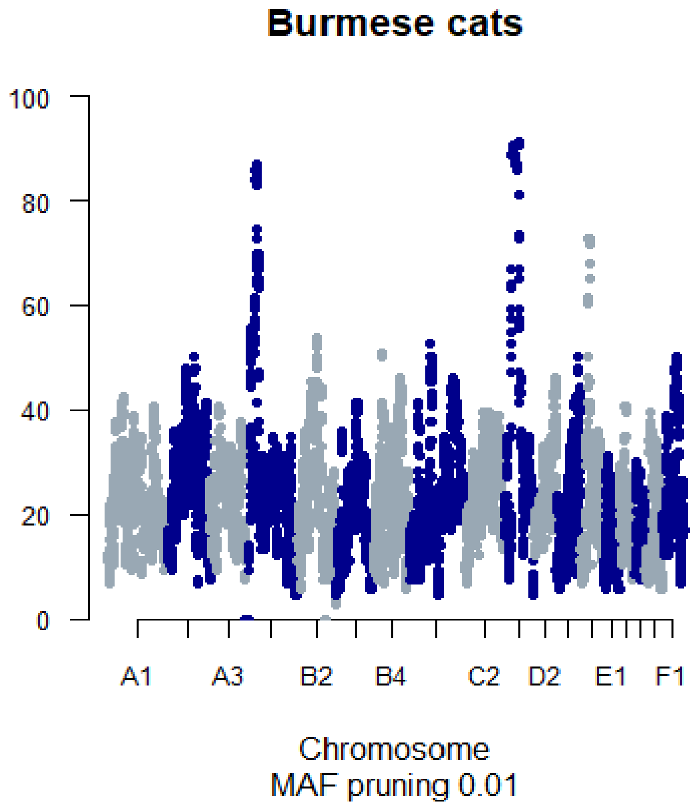
Where ROH_{max} is calculated as the total ROH length of a completely homozygous individual using the current analysis settings.

Correct settings would give ± 100% genome coverage (F_{ROH}=100%)

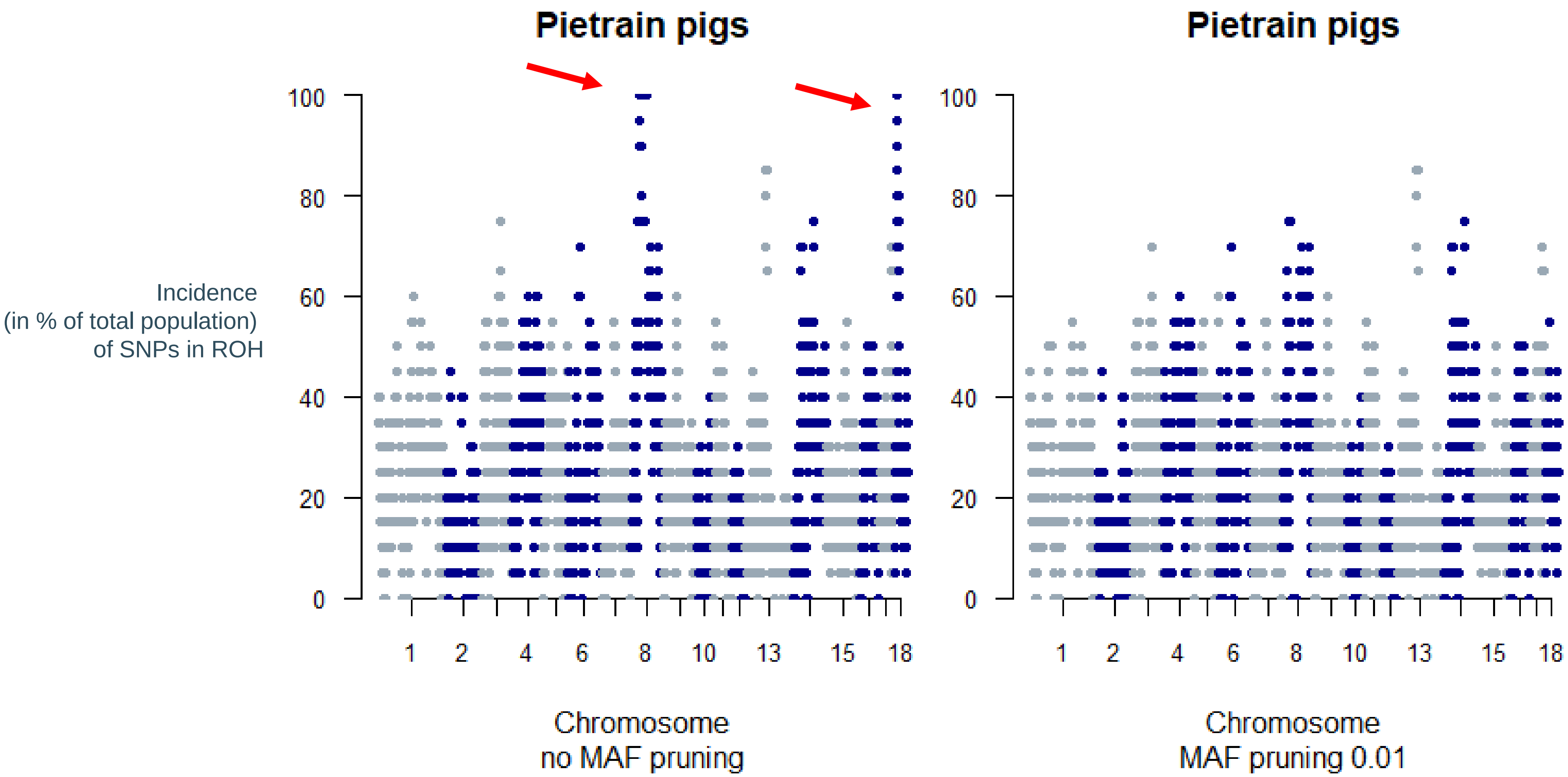
Pruning for low MAF

Discarding SNPs with low minor alleles leads to undetected ROH (islands) and a drop in genome coverage



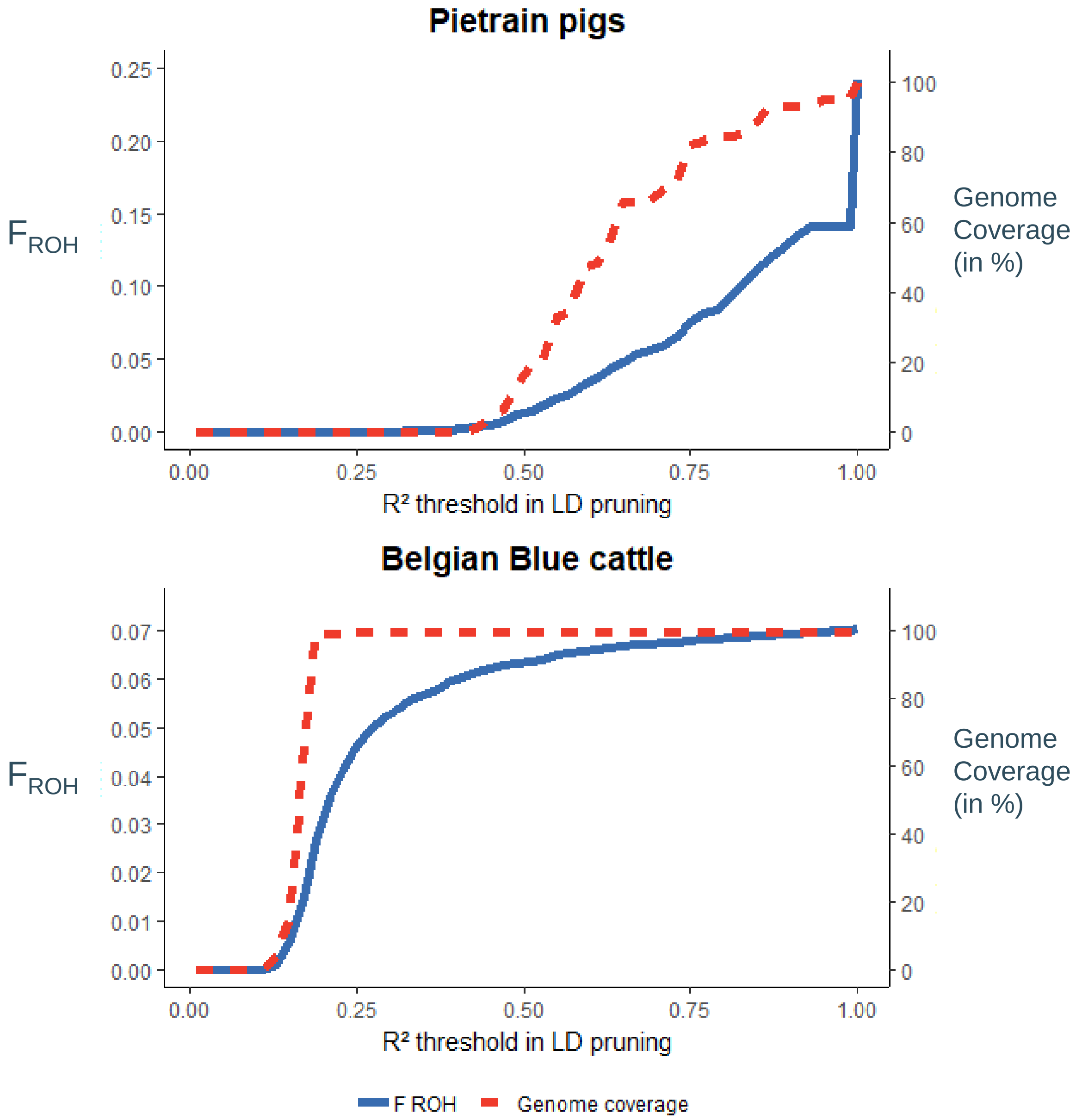


Pruning for low MAF



Pruning for LD

- Implications on ROH detection
- Genome coverage drop
- Effect is population dependent



Pruning for LD and MAF

For GWAS:

- Rare alleles (MAF < 0.05) are of little interest
- Highly correlated SNPs (LD) only slow down the analysis

For ROH detection:

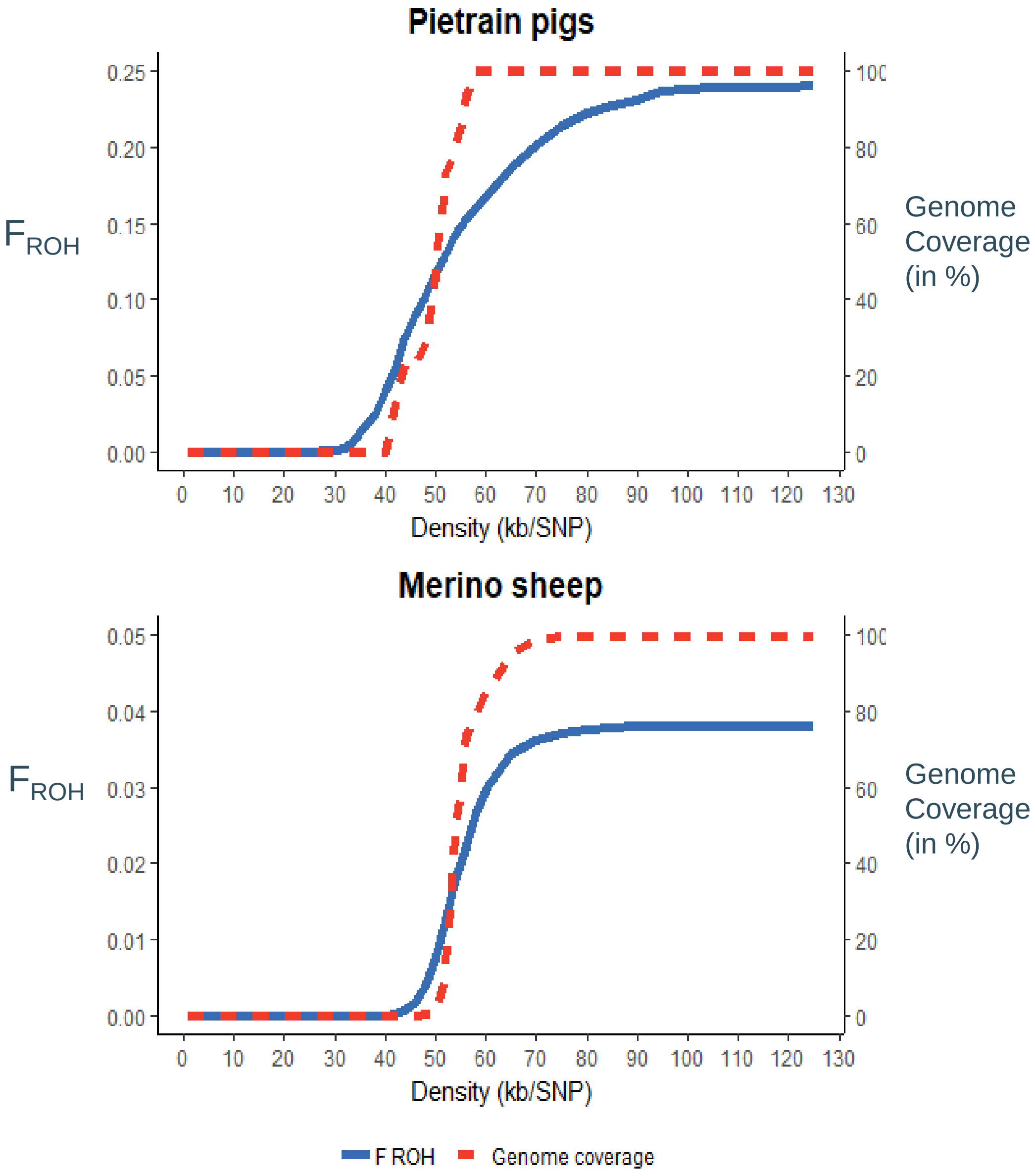
- No harm in including rare alleles
- Computation time is not that critical using medium density genotypes

Minimal SNP Density

- SNP density expressed in minimal $\frac{\mathrm{kb}}{\mathrm{SNP}}$

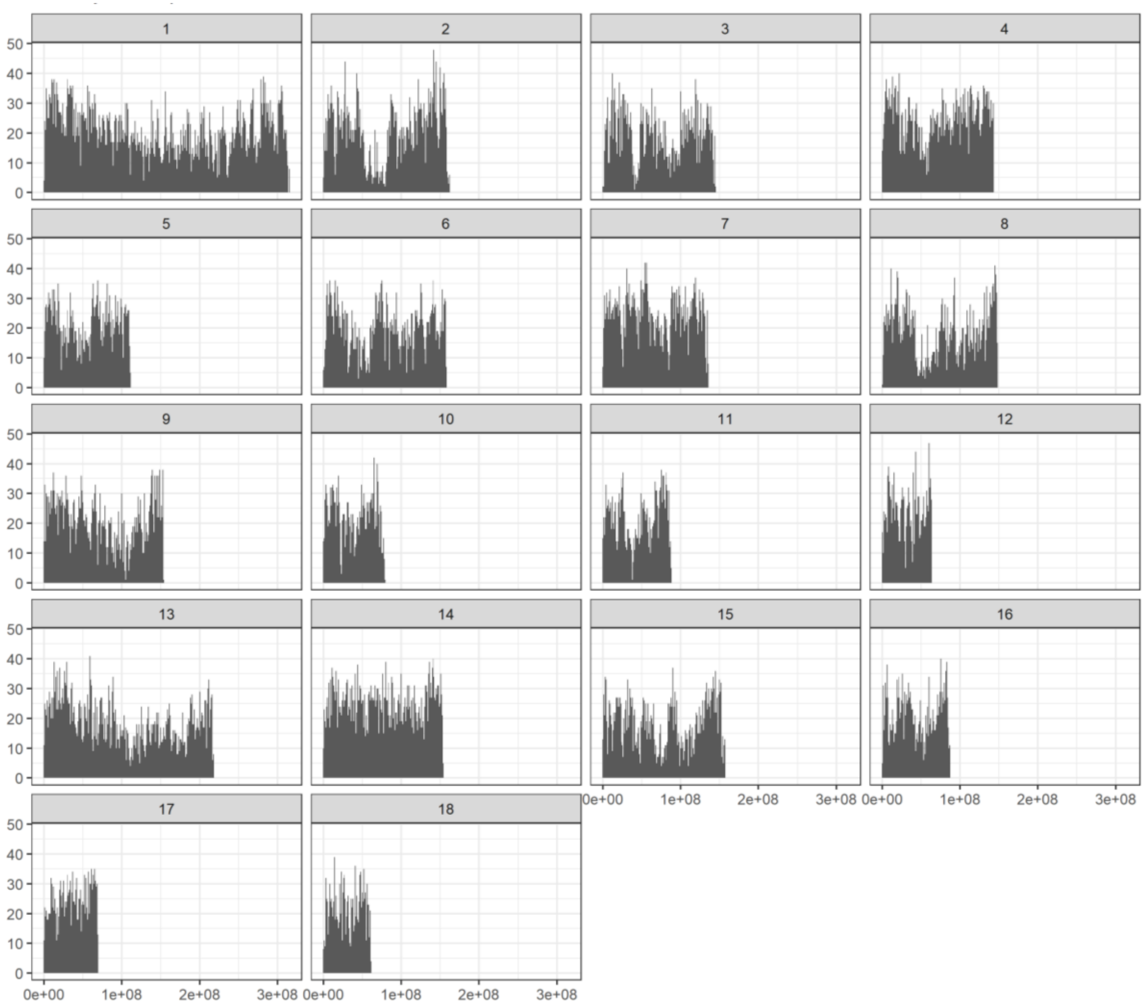
- Default PLINK setting is minimal 50 $\frac{kb}{SNP}$

- Population and array dependent



Local SNP density differences

SNP density (in # SNPs/Mb)



Location in the genome

Conclusions

- MAF and LD pruning affects ROH detection and is perhaps unnescessary in ROH detection
- Low minimal density (in kb/SNP) can lead to low genome coverage
- Calculating genome coverage helps to detect problems
- Report all PLINK settings in your publications

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Complete results and discussion are reported in
Meyermans & Gorssen et al., under review with BMC Genomics

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Thank you for your attention

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Minimal ROH length (SNP)

The minimal number of SNPs in a ROH was determined by the formula proposed by Lencz et al. and adapted by Purfield et al.:

$$L = \frac{log_e \frac{\alpha}{n_s n_i}}{log_e(1 - het)},$$

with n_s the number of genotyped SNPs per individual, n_i the number of genotyped individuals, α the percentage of false positive ROH (0.05) and het the mean heterozygosity across all SNPs.