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> Large scale screening for genetic defects in Holstein cattle using transmission disequilibrium test

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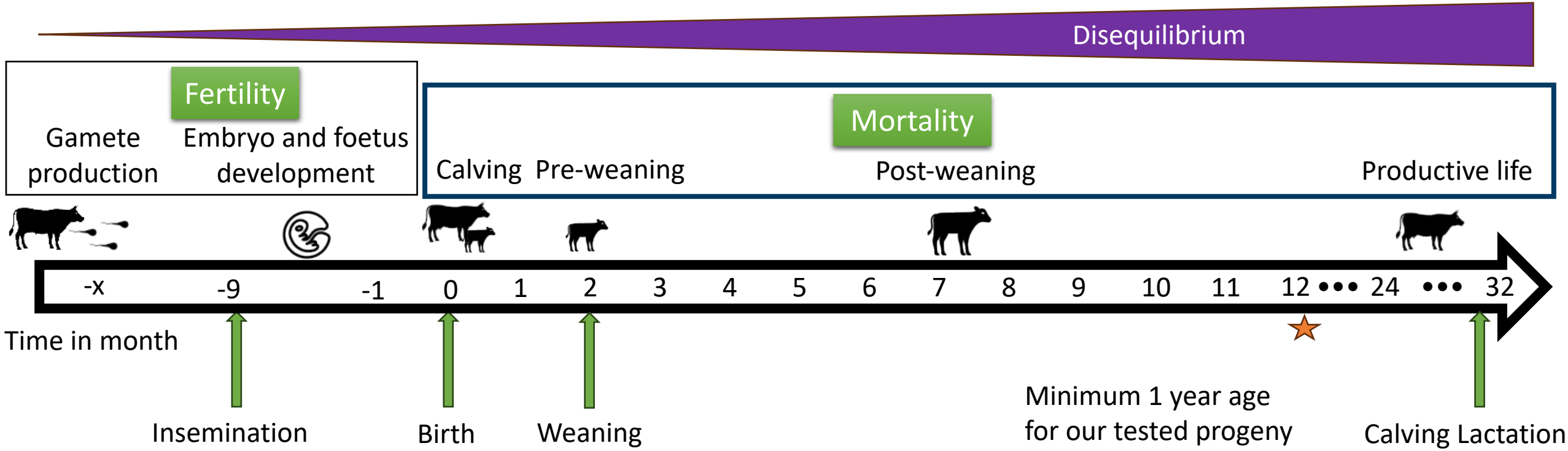
IDELE and INRAE

Background

- Project funded by APIS-GENE, IDELE, ANRT and with supervision of INRAE

- Program aiming to reduce juvenile mortality in the dairy cattle population.

- 12% mortality before 365 days for female calves of Holstein population

- Various reasons, mainly related to dystocia, intestinal and pulmonary infections

- The bovine national genetic database have 2.5 million genotypes and around 8000 sequences

How can we use the national genetic database to understand some of the causes of mortality in the Holstein population?

Potential causes of disequilibrium in the progeny from a bull

Disequilibrium

Fertility

Gamete production

Embryo and foetus development

Mortality

Calving   Pre-weaning

Post-weaning

Productive life

| -x | -9 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | ••• | 24 | ••• | 32 |

Time in month

Insemination

Birth

Weaning

Minimum 1 year age for our tested progeny
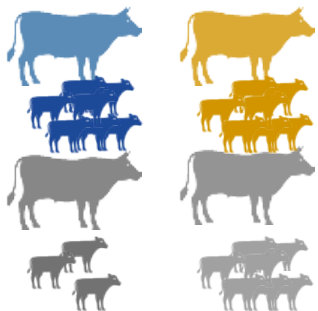
Calving Lactation

- Mendelian law of equal segregation:
  Random transmission of alternative alleles at a diploid locus to gametes and progeny (1)
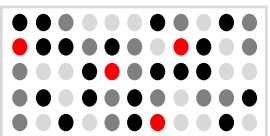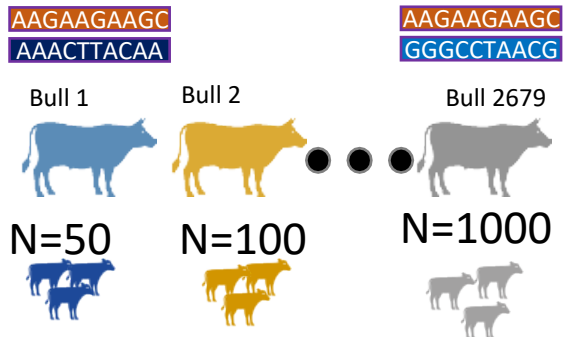- There are exceptions to this law, leading to disequilibrium in transmission to the progeny.

Number of genotypes

TDT method

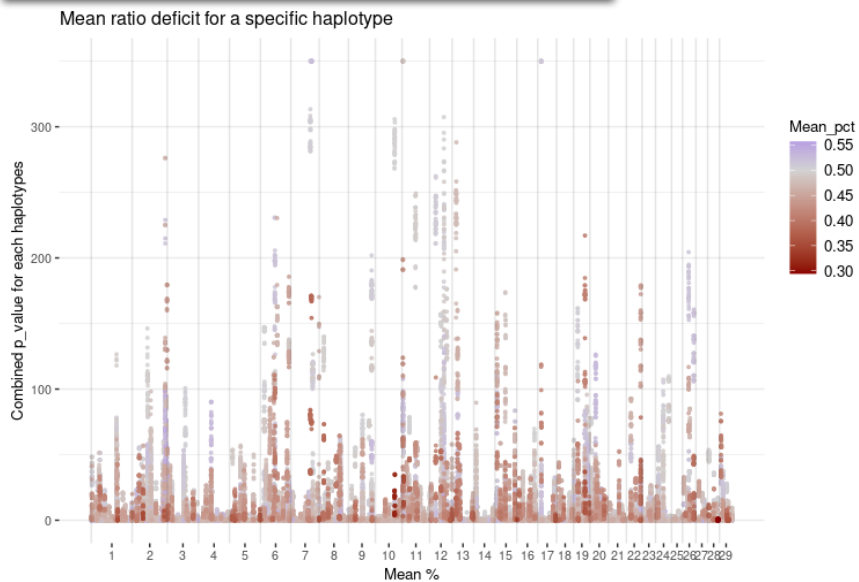## 1. Selection of data

>50 calves genotyped / sire

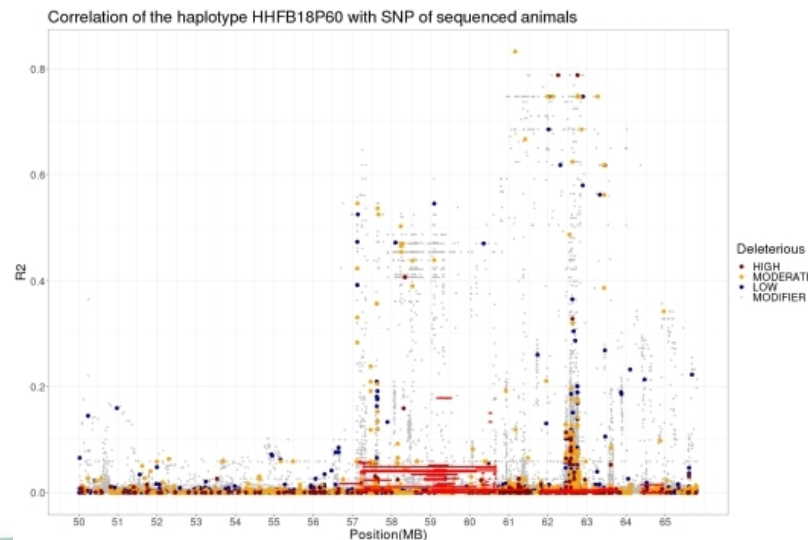Selection of good quality SNP

2. Transmission disequilibrium test

AAGAAGAAGC
AAACTTACAA

AAGAAGAAGC
GGGCCTAACG

Bull 1   Bull 2   Bull 2679

N=50   N=100   N=1000

AAGAAGAAGC	NNNNNNNNN
Hap tested	Other
12	38
33	67
•••	•••
250	750

Chi^2 test for 1.7 Mo haplotypes

3. Selection of haplotypes

Mean ratio deficit for a specific haplotype
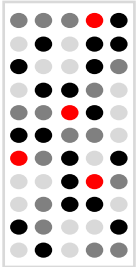
4. Sequence correlation

Correlation of the haplotype HHFB18P60 with SNP of sequenced animals

1) Selection of data

- Selection of 2,483 bulls with at least 50 female calves genotyped

- Lead to a dataset of 1,106,449 genotyped calves

- Selection of 44,596 SNP from a 53,469 SNP chip:
  - Autosomal
  - MAF
  - LD analysis
  - Reliability

- Genotypes were imputed and phased as part of the routine evaluation (Fimpute)

- Definition of 20 SNP haplotypes

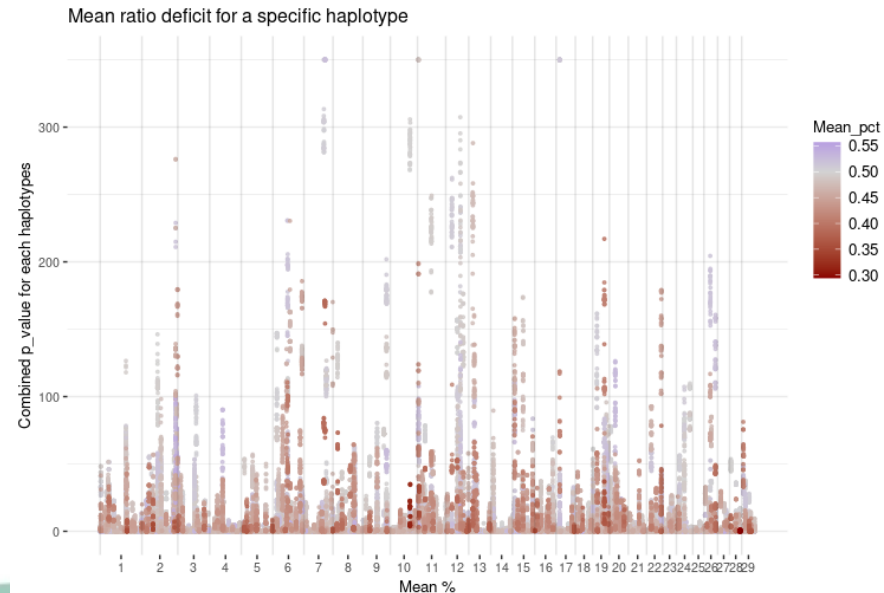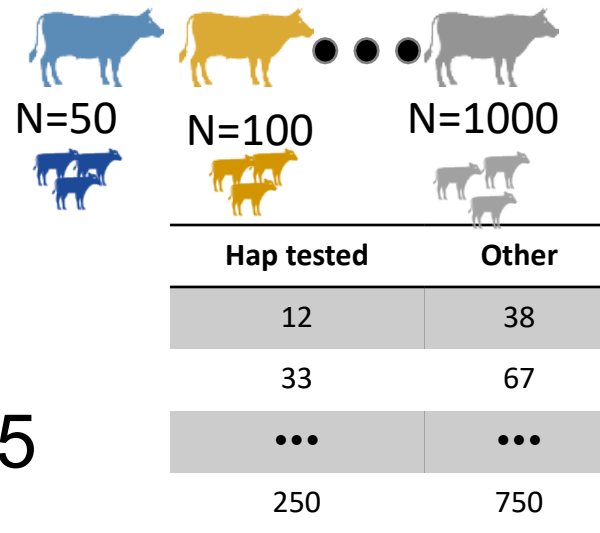1. Selection of data

2. Transmission disequilibrium test

3. Selection of haplotypes

4. Sequence correlation

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Transmission disequilibrium tests

2) Transmission disequilibrium test

- In each progeny group, compute the transmission ratio of each haplotype

- Use of goodness-of-fit tests

- Test each haplotype with at least 5 carrier sires

- 1.7 million haplotypes tested

- 28,601 haplotypes in significant disequilibrium

N=50   N=100   N=1000

Hap tested	Other
12	38
33	67
...	...
250	750

Mean ratio deficit for a specific haplotype

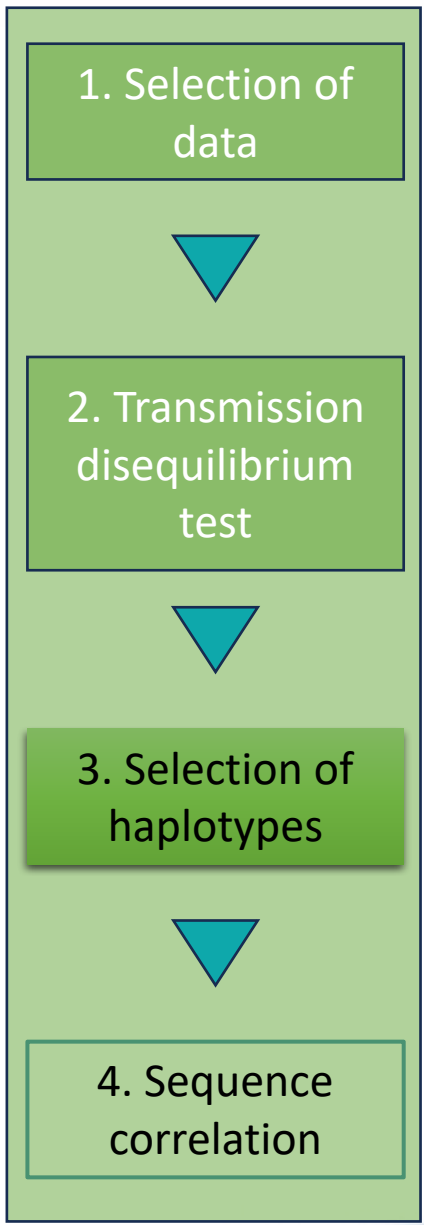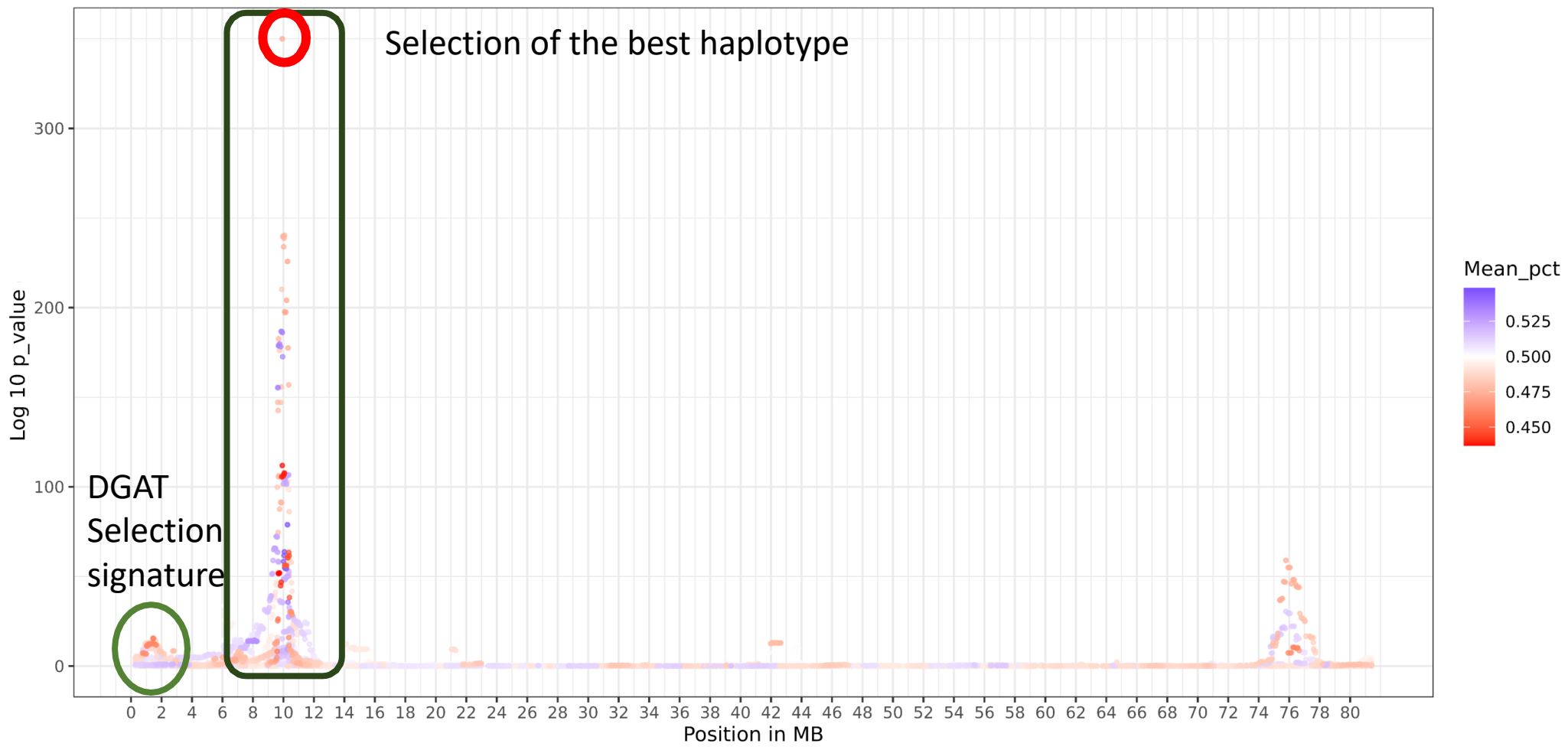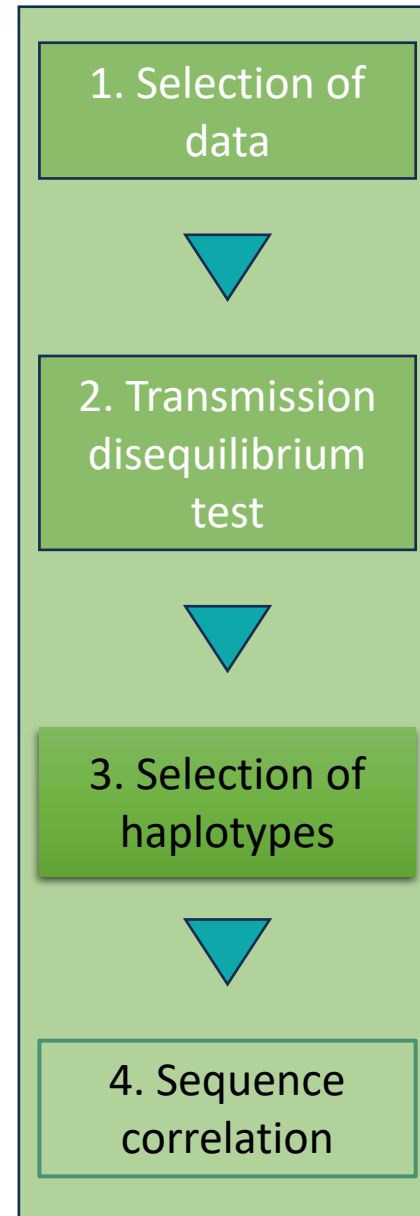| 1. Selection of data |
|---|
| 2. Transmission disequilibrium test |
| 3. Selection of haplotypes |
| 4. Sequence correlation |

3) Selection of haplotypes

BTA: 14, Log10 p_value of chi2 test between phases for allelic disequilibrium

Selection of the best haplotype

DGAT Selection signature

Mean_pct
- 0.525
- 0.500
- 0.475
- 0.450

1. Selection of data

2. Transmission disequilibrium test

3. Selection of haplotypes

4. Sequence correlation

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Transmission disequilibrium tests
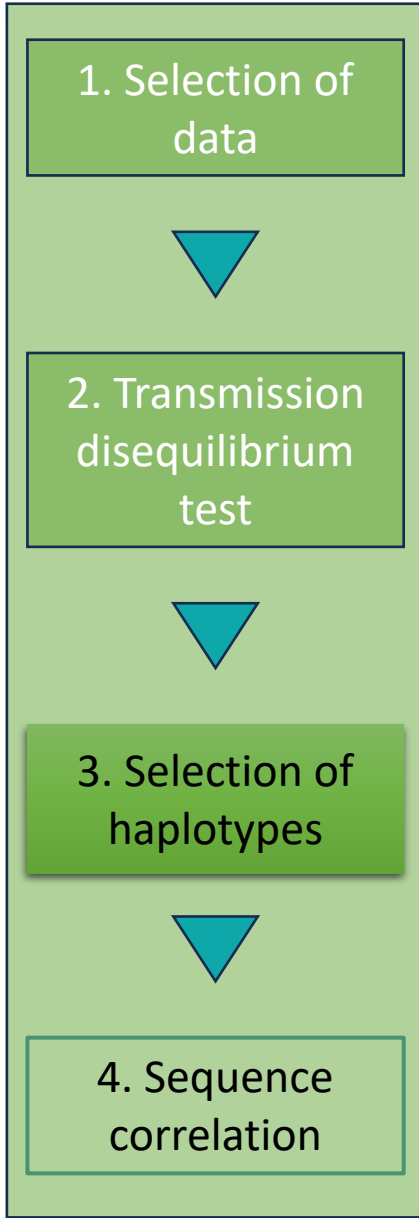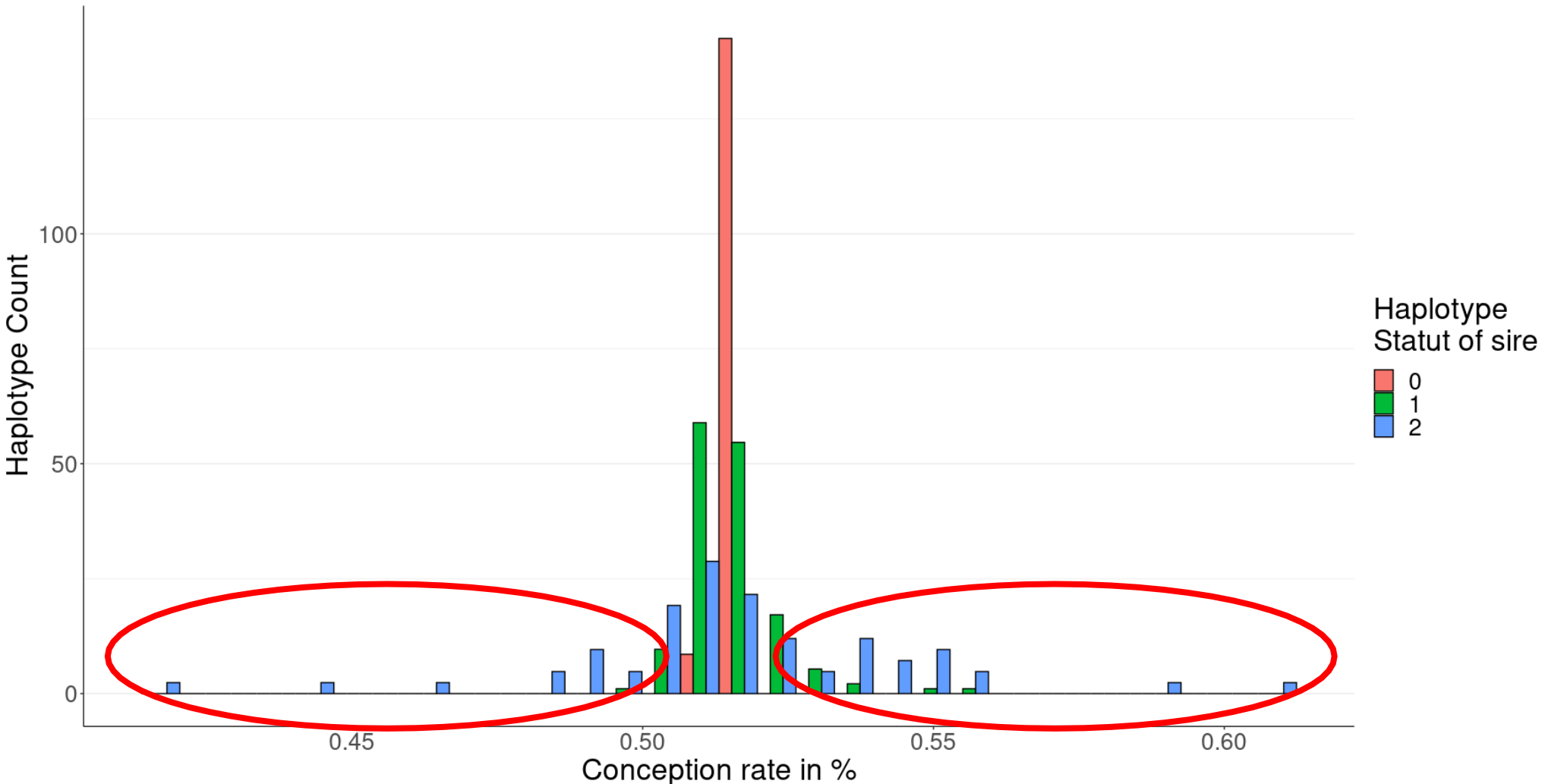74th EAAP Annual Meeting 28_08_2023/ Besnard Florian

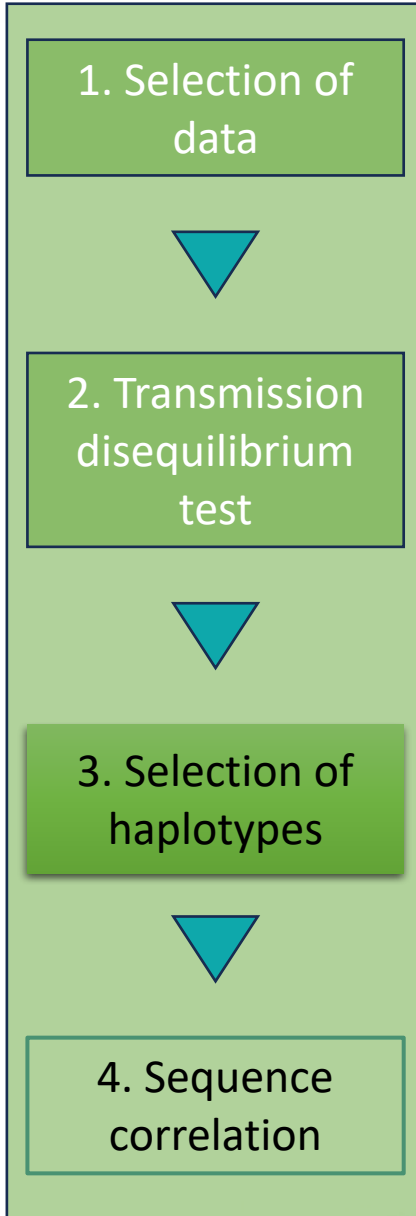# 3) Effects of haplotypes in mortality and conception

- 140 haplotypes were selected
- All animals genotyped were given a statut for each haplotype
- Selection of all inseminations after 2010 and all calves born with sire genotyped
- We tested the effect of each haplotype both on insemination and on born calves
- Mean conception rate of our population is 51%
- Mean mortality rate is 12.1% at one year

1. Selection of data

2. Transmission disequilibrium test

3. Selection of haplotypes

4. Sequence correlation

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Transmission disequilibrium tests

3) Haplotype effects → Conception rate

Transmission disequilibrium tests

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3) Haplotype effects → Mortality rate at 1 year

Transmission disequilibrium tests

74th EAAP Annual Meeting 28_08_2023/ Besnard Florian
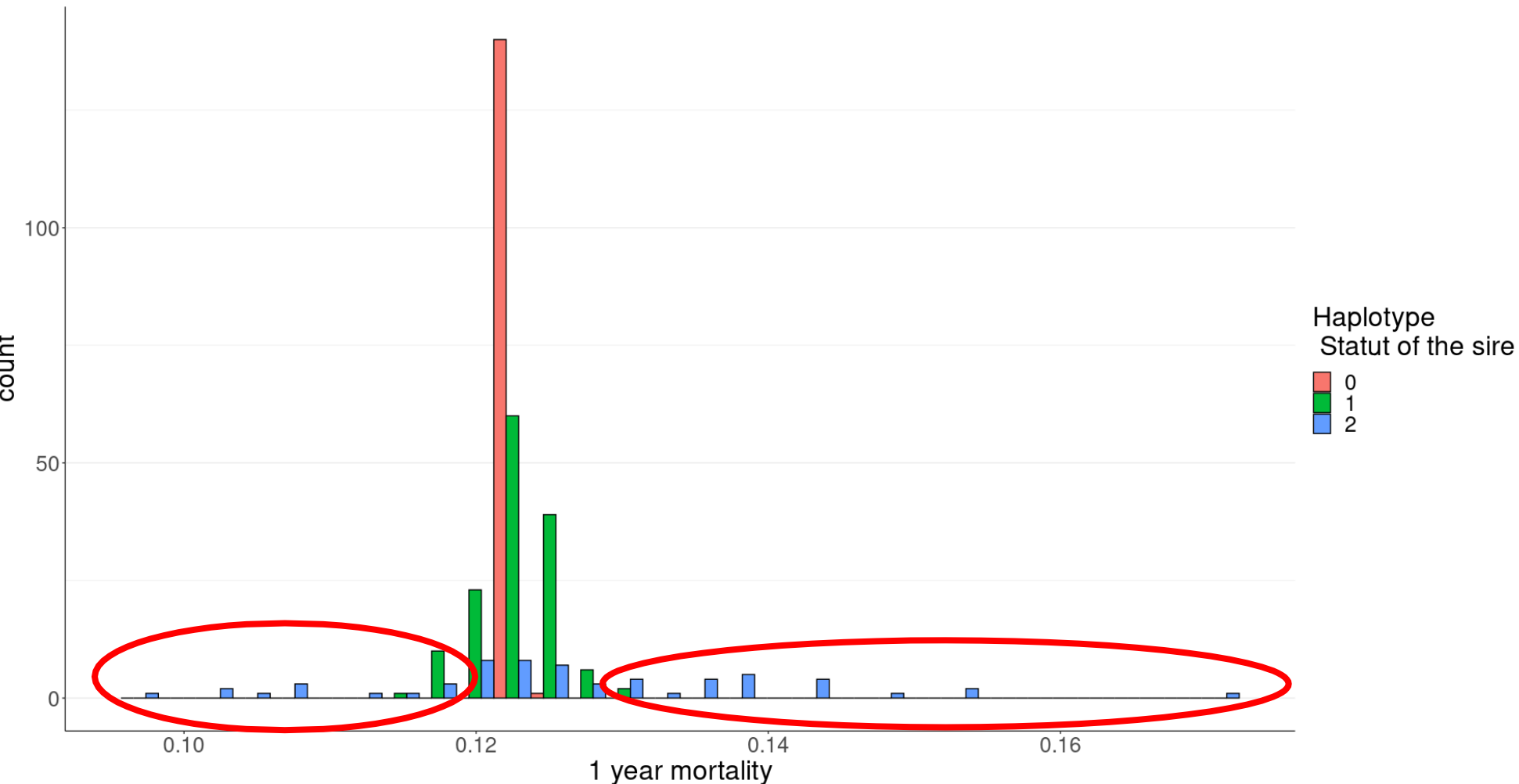
3) Example of Outliers

Conception rate

Sire Statut	Haplo	Conception rate	NB insemination	Mean birthdate
2	HHFB12P46	41.94%	32118	2015
2	HHFB17P66	44.37%	2132	2019
2	HHFB28P4	46.29%	1255	2012
2	HHFB2P135	48.41%	5224	2019
2	HHFB6P37	48.61%	10513	2012
2	HHFB20P30	48.67%	52842	2018
2	HHFB29P49	61.13%	3913	2011
2	HHFB8P79	58.97%	2374	2011
2	HHFB20P12	55.65%	5450	2012

Mortality at one year

Sire statut	Haplo	Dead 1y	NB	Mean birthdate
2	HHFB9P90	17.15%	3129	2021
2	HHFB7P69	15.26%	12055	2016
2	HHFB29P13	15.24%	1706	2015
2	HHFB12P86	14.65%	3434	2019
2	HHFB18P60	14.38%	9390	2020
2	HHFB6P110	14.35%	3178	2017
2	HHFB15P41	9.66%	6120	2022
2	HHFB29P29	10.27%	1392	2011
2	HHFB8P79	10.30%	1602	2014
2	HHFB12P24	10.53%	1674	2016
2	HHFB23P35	10.68%	10233	2017
2	HHFB20P12	10.78%	2347	2014

- Mean Conception rate of 51%
- Mean mortality rate at 1 year of 12%

1. Selection of data

2. Transmission disequilibrium test
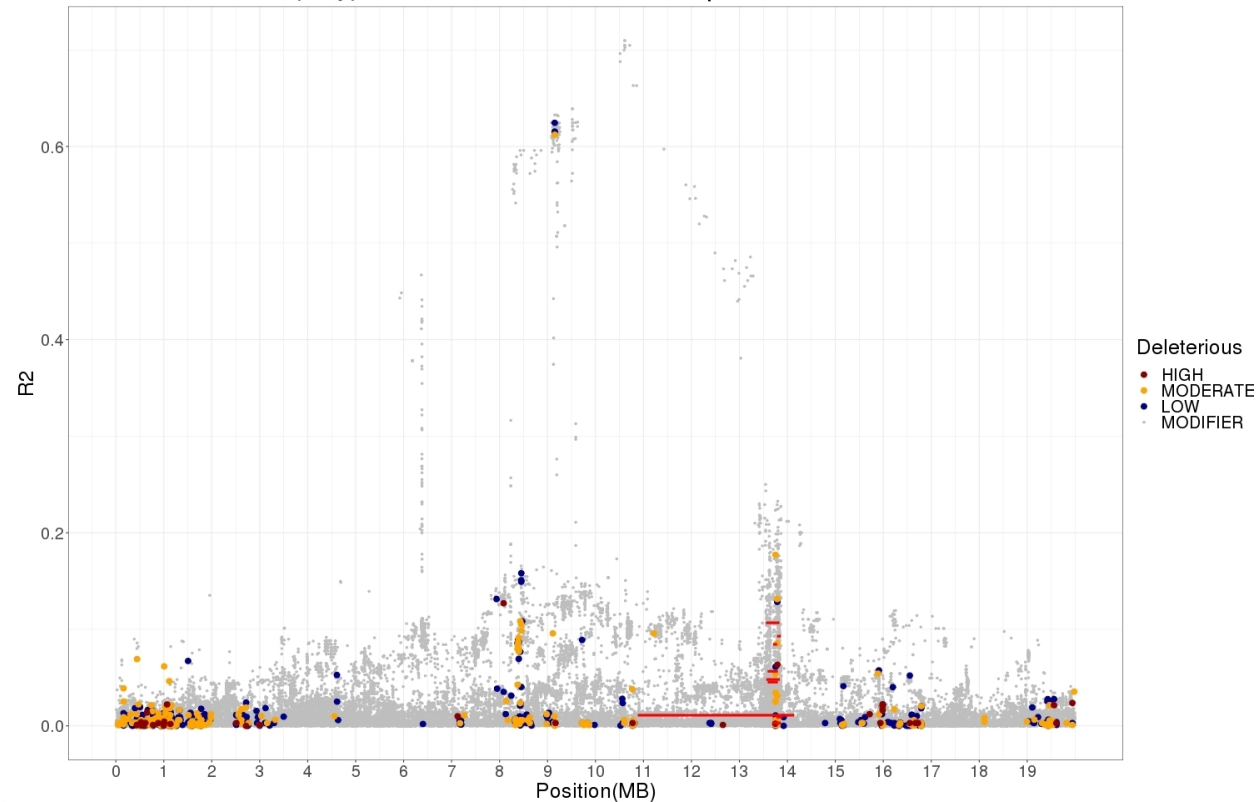
3. Selection of haplotypes

4. Sequence correlation
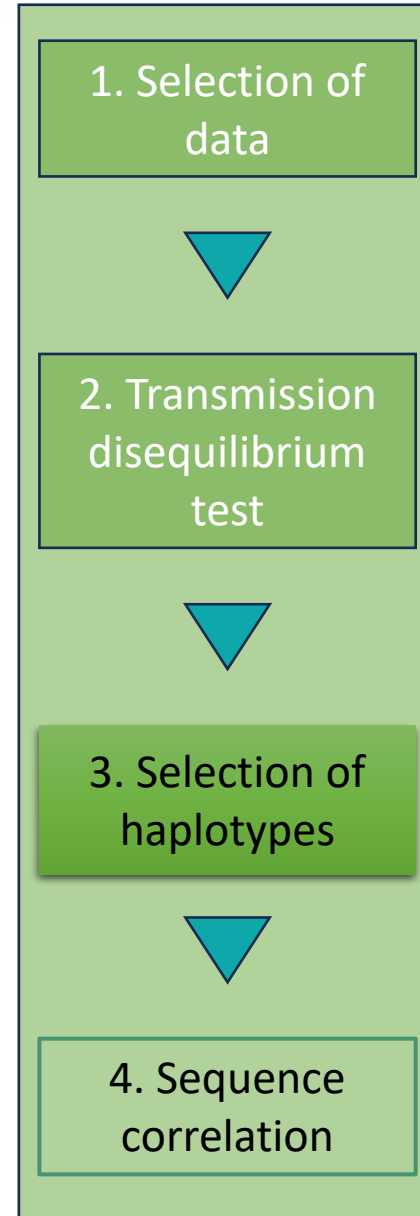
4) Sequence correlation

- 3000 bulls with both genotypes and sequences
- Correlation of haplotype with WGS information over +- 10 MB around the haplotype peak

- Correlation with:
  - Structural variants
  - SNP
- VEP annotation:
  - SIFT Score

Correlation of the haplotype HHFB14P10 with SNP of sequenced animals

Deleterious
- HIGH
- MODERATE
- LOW
- MODIFIER

1. Selection of data

2. Transmission disequilibrium test

3. Selection of haplotypes

4. Sequence correlation

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Discussion and conclusion

- This method detected more than 140 haplotypes in significant transmission disequilibrium.

- This method can facilitate the detection of anomalies associated with fertility and mortality, while also enabling the identification of signature selection.

- Correlation with sequences SNP and SV help pinpoint candidate variants for those haplotypic distorsions.

- The method used is strongly impacted by poor quality markers, from initial chip calling to imputation errors.

- Real cases studies are required to validate the best candidate variants.

Thank you for your attention

Any questions?

High mortality haplotype HHFB9P90

BTA: 9, Log10 p_value of chi2 test between phases for allelic disequilibrium