Development of a Genetic Marker Panel to Predict Reproductive Longevity in Holstein Cattle

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Reproductive Longevity

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Reproductive Longevity (RL) is the result of a complex of traits involving longevity, ovarian function, fertility, stress resistance (coping with the stress of lactation and reproduction), robustness and health. It is reasonable to assume that a relatively large number of genes and pathways are involved and that the genetic bottlenecks overlap and differ between mouse and cattle.

Reproductive Longevity

RL is affected by a lot of different traits, some of which are low in heritability...

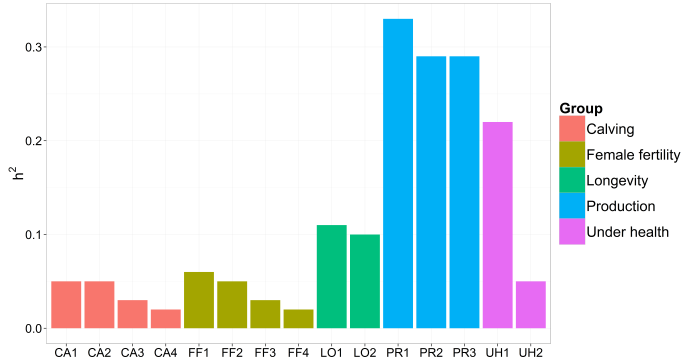

Fig. The average cattle heritability for *Interbull* countries across selected traits (official information, 2015).

The aim of the study

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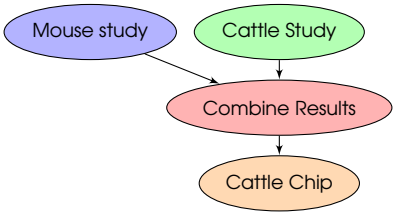
- identification of candidate genes associated with RL at different level of mouse and cattle study:
  - low density genome resolution,
  - high density genome resolution,
  - transcriptome,
- commercial DNA marker-based tests of RL for the Holstein cattle industry.



Mouse study

- Material
- Three studies: microarray, poolseq and rnaseq

Material, Unique mouse resource

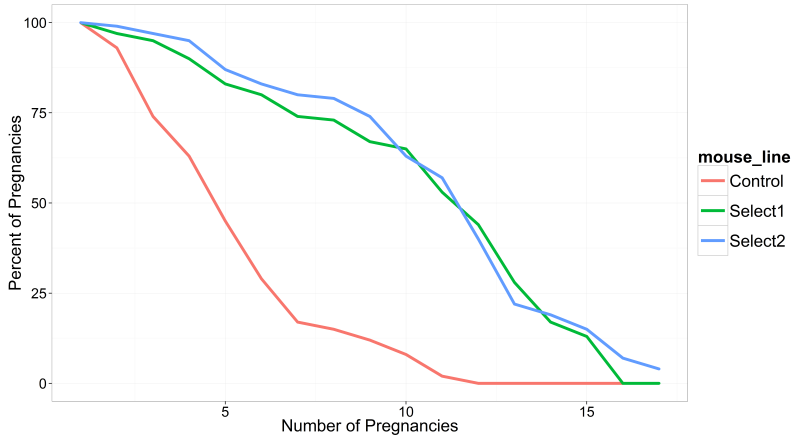
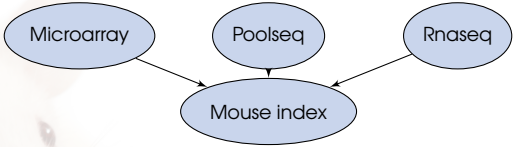


Fig. Effect of selection on number of lifetime pregnancies (Don Crober et al., 2007).

Methods and results



- contrast between select and control lines,

8 pooled samples, mean coverage 50x per pool,
Poolseq analysis based on Cochran-Mantel-Haenszel statistic,
after filtration 6.4M of SNPs,
gene selection based on modified TopQ method described by
580K highly significant SNPs within 13.4K genes,
two additional contrasts: ovary vs. pituitary tissue and reproductive vs.
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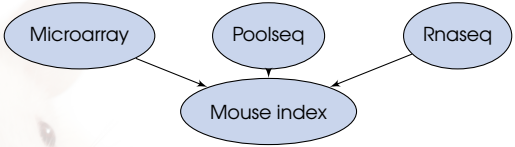
Methods and results



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Whole genome sequencing approaches (Poolseq)

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- new method of cost-effectiveness sequencing,

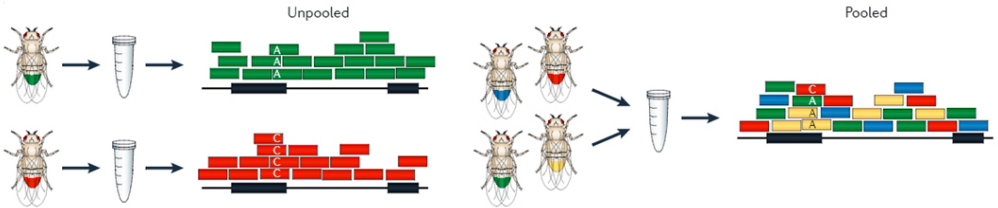
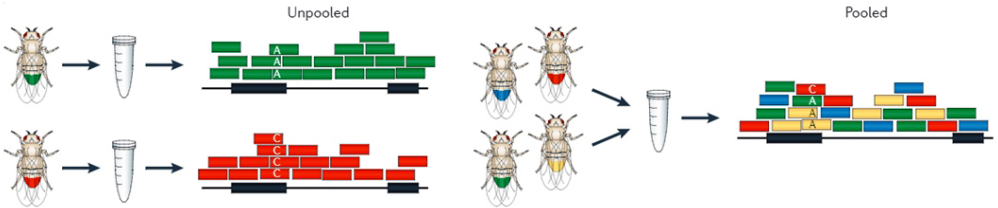


Fig. Comparison of sequencing strategies (Schlötterer et al., 2014).

Whole genome sequencing approaches (Poolseq)

- new method of cost-effectiveness sequencing,
- similar to reduced representation library, strategy used to discover large numbers of genome-wide SNP with high MAF (Matukumalli et. al 2009).



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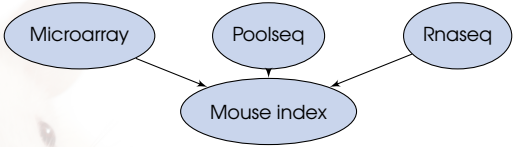
Methods and results, continue



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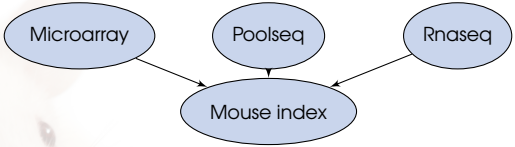

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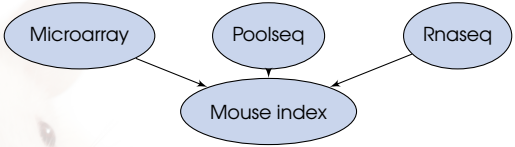

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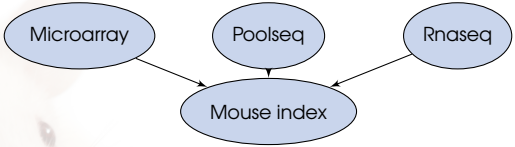

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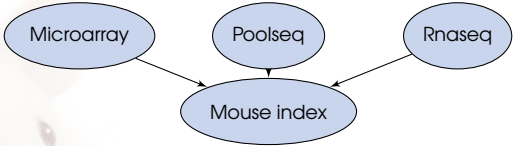

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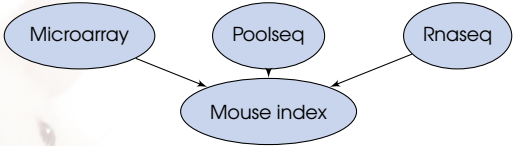

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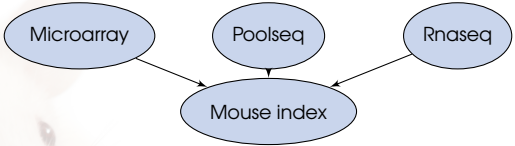

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- differential expression analysis.

Cattle study

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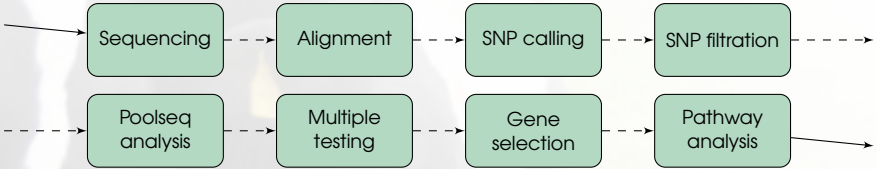
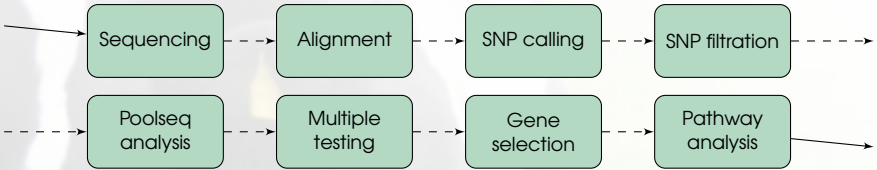
- Poolseq supported by individual sequencing study

Methods, Poolseq



- 16 pools with high and 16 pools with low longevity bulls,

Methods, Poolseq



- 16 pools with high and 16 pools with low longevity bulls,
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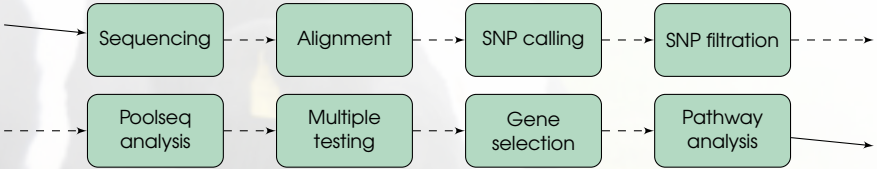
Methods, Poolseq



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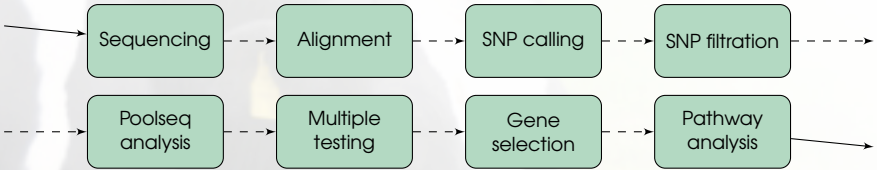
Methods, Poolseq



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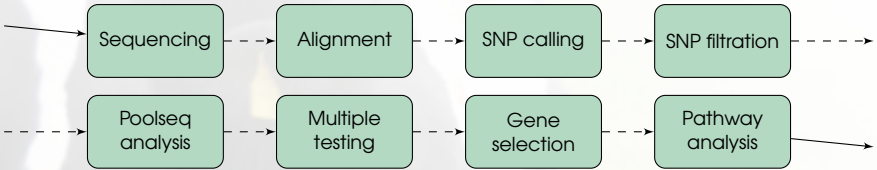

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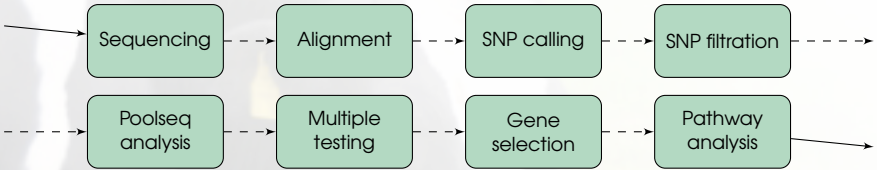

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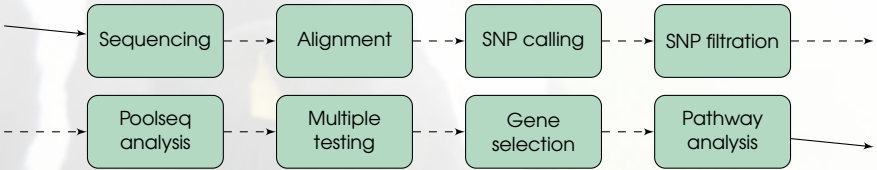
Methods, Poolseq



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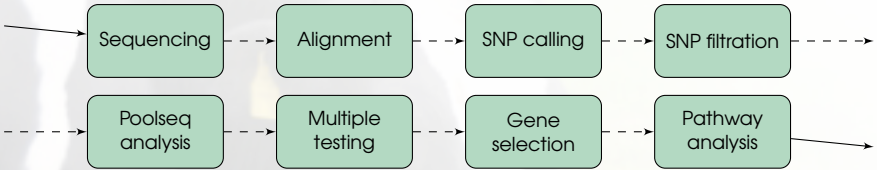

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Results, Poolseq

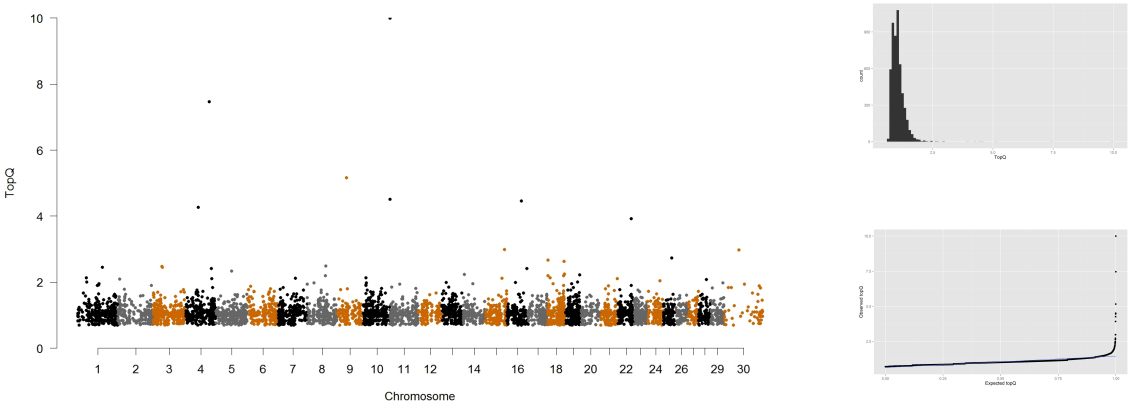

Fig. Manhattan plot for TopQ (left) with histogram (right-top) and qq (right-bottom) plots for all coding genes.

Results, Meta-analysis

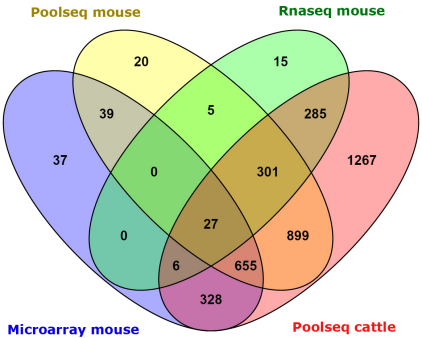

Fig. Venn plot representing significant genes across considered studies based on cattle annotated genes.

- homology issue,
  - 45K for mouse and 24K cattle genes in annotation table,
- more than 3300 significant genes across studies based on cattle annotation table,
- low correlation between studies,
- weighted index used to rank genes,
- more than 1400 significant genes which were useful for cattle,
  - with more than one significant SNP within gene.

SNP panel selection

- SNP selection algorithm based on significance level and linkage disequilibrium,
- testing panel with more than 1400 genes and QTLs (Zhi-Liang et al., 2013),
- testing panel with more than 4500 SNPs,
- SNP classes: within gene, within QTL, intergenomic SNPs.

Conclusions and further plans

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Conclusions:

- multidisciplinary approach,
- testing panel in production, Illumina approach.

Further plans:

- many levels of testing: individual SNP, gene, genomic selection approach.
- validation procedure:
  - more than 3000 bulls,
  - stand alone panel,
  - combination with Illumina Bovine50K.

Acknowledgements

Jyoti Joshi, Nehil Jain, Robert Beiko,
Hein van der Steen and people from Lab...

Erik Mullaart and Chris Schrooten
Barry Simpson and Stewart Bauck

NRC-IRAP
ACOA
Mitacs Accelerate

Thank you for your attention

- Questions?

Are you interested in panel utilization?
Hein van der Steen, hein.vandersteen@btinternet.com

genewise R package

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- high performance R package dedicated for GWAS and Poolseq data,
- utility functions, SNP selection based on multiple testing, mapping SNPs to genes, genewise statistics for gene ranking, annotation,
- SNP selection algorithm to produce Illumina microarray design,
- summary statistics for different steps to give control to the user,
- package is built using `data.table`, `dplyr family`, `qvalue`, `multtest`, `ggplot2`.

https://github.com/nehiljain/genewiseR

Mouse study

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- Material

Mouse study

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- Material
- Microarray

Mouse study

- Material
- Microarray
- Poolseq

Mouse study

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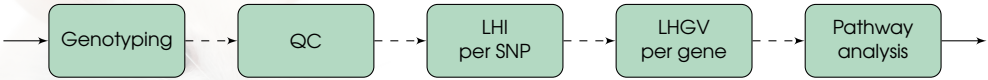
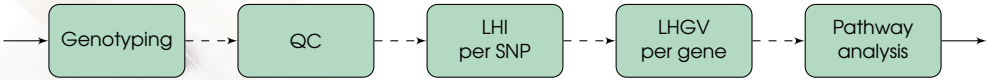
- Rnaseq

Methods, Microarray



- select (S) and control (C) lines with more than 350 samples,

Methods, Microarray



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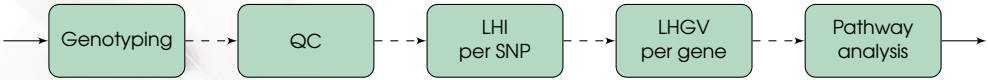

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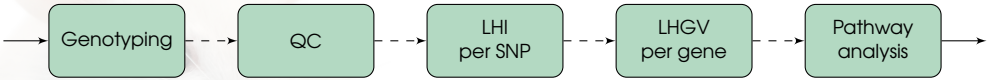

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- Used LHI results to calculate LHGV per gene.

Results, Microarray

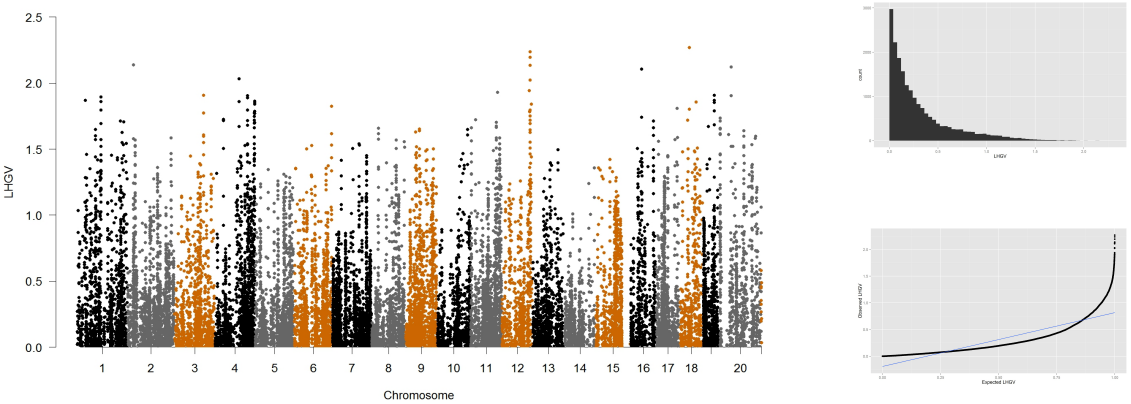
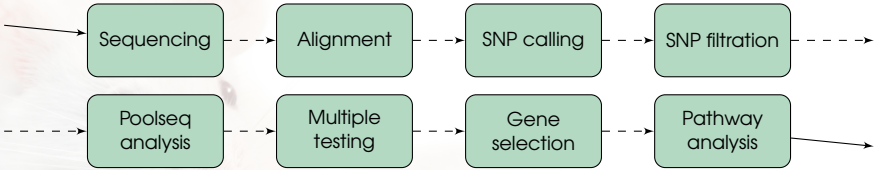


Fig. Manhattan plot for LHGV (left) with histogram (right-top) and qq (right-bottom) plots for protein coding genes.

Methods, Poolseq



- 4 select and 4 control pools with more than 20 individuals per pool,

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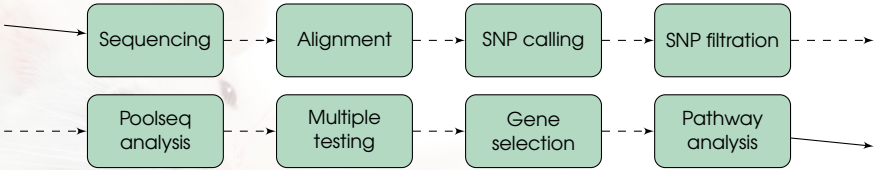

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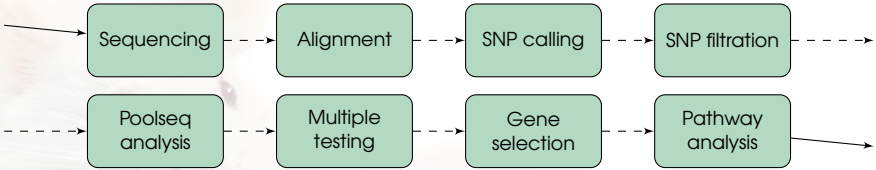

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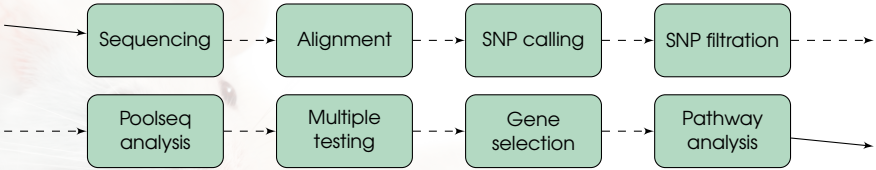

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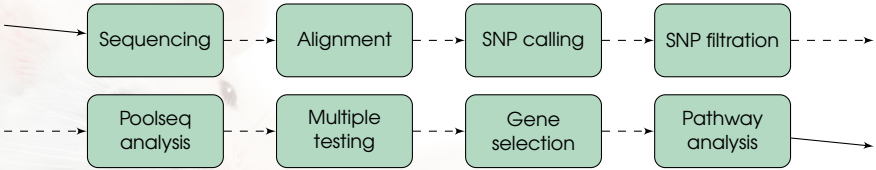

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Results, Poolseq

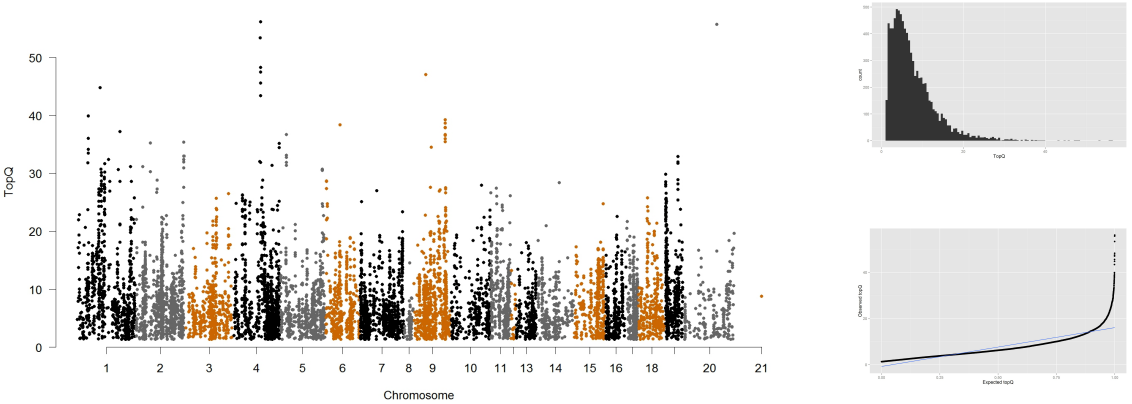
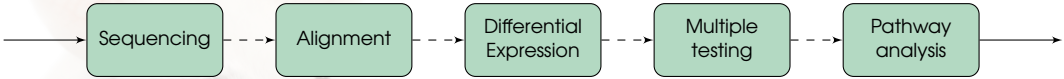


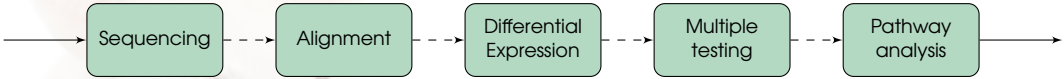
Fig. Manhattan plot for TopQ (left) with histogram (right-top) and qq (right-bottom) plots for protein coding genes.

Methods, Rnaseq



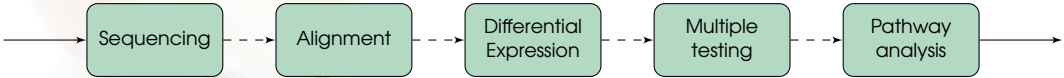
- 20 pools with more than 20 individuals per pool,

Methods, Rnaseq



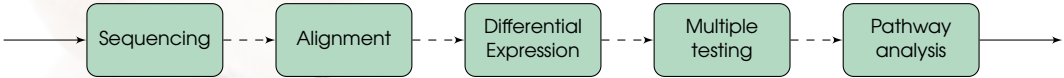
- 20 pools with more than 20 individuals per pool,
  - more than 163M reads per pool (MAPQ30),

Methods, Rnaseq



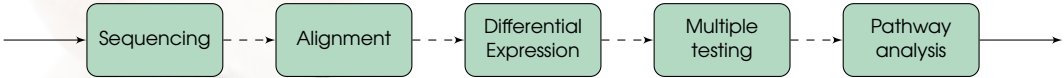
- 20 pools with more than 20 individuals per pool,
  - more than 163M reads per pool (MAPQ30),
- 3 different contrasts:

Methods, Rnaseq



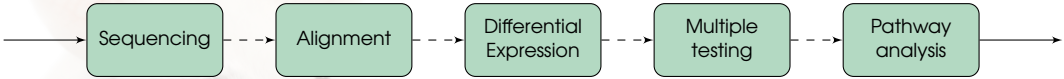
- 20 pools with more than 20 individuals per pool,
  - more than 163M reads per pool (MAPQ30),
- 3 different contrasts:
  - select and control,

Methods, Rnaseq



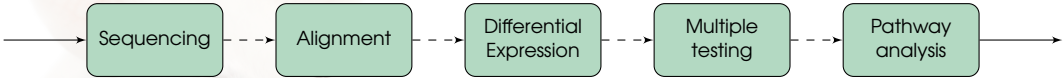
- 20 pools with more than 20 individuals per pool,
  - more than 163M reads per pool (MAPQ30),
- 3 different contrasts:
  - select and control,
  - ovary and pituitary tissue,

Methods, Rnaseq



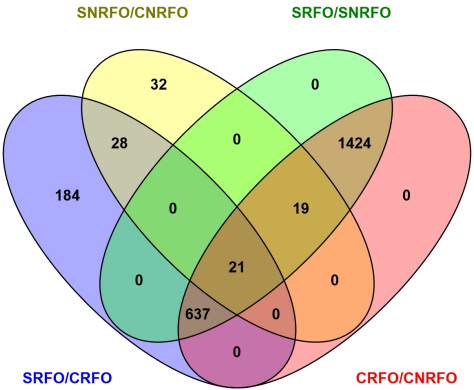
- 20 pools with more than 20 individuals per pool,
  - more than 163M reads per pool (MAPQ30),
- 3 different contrasts:
  - select and control,
  - ovary and pituitary tissue,
  - reproductive and non-reproductive females,

Methods, Rnaseq



- 20 pools with more than 20 individuals per pool,
  - more than 163M reads per pool (MAPQ30),
- 3 different contrasts:
  - select and control,
  - ovary and pituitary tissue,
  - reproductive and non-reproductive females,
- differential expression analysis with utilization DESeq and edgeR Bioconductor R packages.

Results, Rnasea



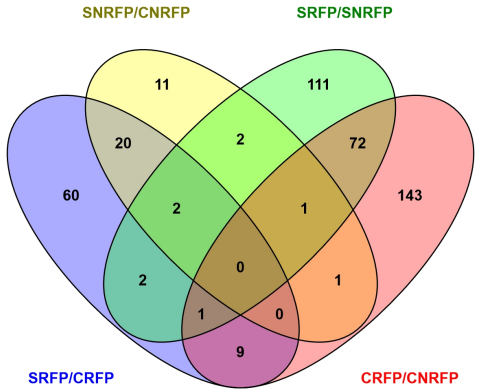


Fig. Venn plots for highly significant genes representing different contrasts for ovary (left) pituitary (right) tissue.

Results, Rnaseq

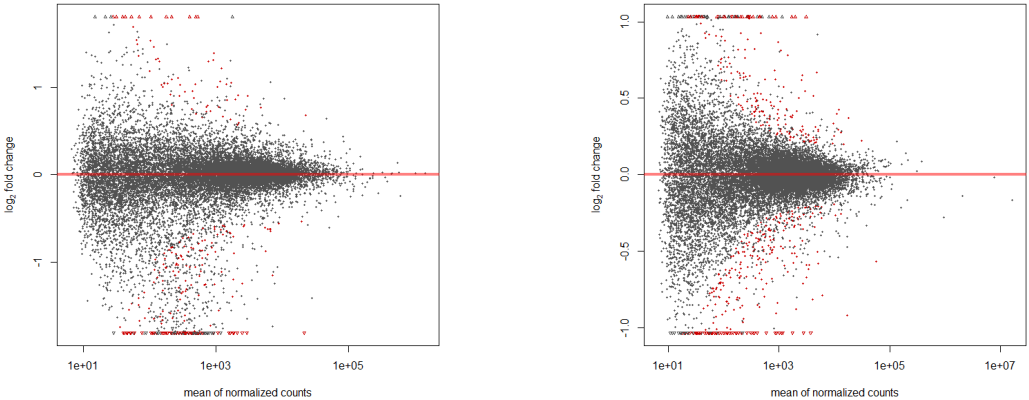
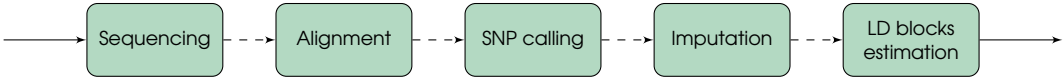


Fig. Plot of normalized mean versus $\log_2$ fold change for the contrast S vs. C for ovary (left) pituitary (right) tissue.

Methods, WGS



- individual WGS,

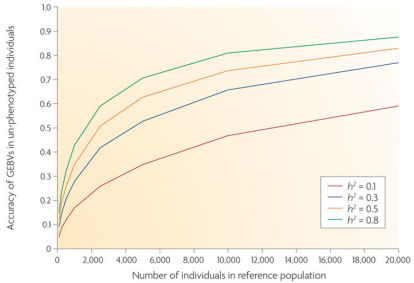
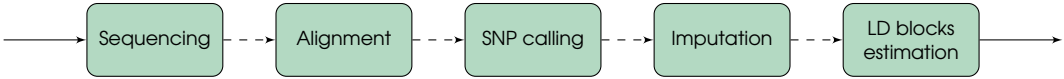
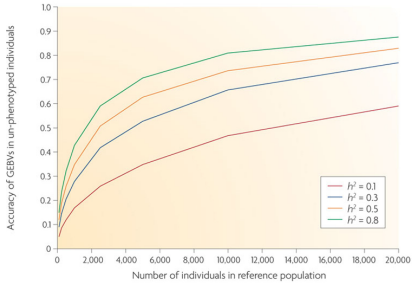


Fig. Accuracy of GEBVs of un-phenotyped individuals (Goddard and Hayes, 2009).

Methods, WGS



- individual WGS,
- 126 bulls with high and low EBVs for longevity,



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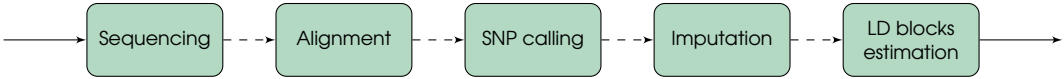
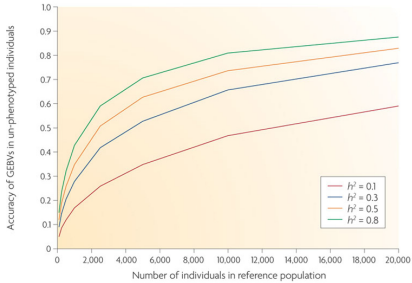


- individual WGS,
- 126 bulls with high and low EBVs for longevity,
- mean coverage 1.5x per individual bull,



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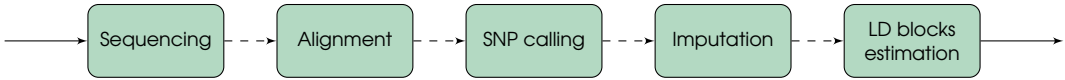
Methods, WGS



- individual WGS,
- 126 bulls with high and low EBVs for longevity,
- mean coverage 1.5x per individual bull,
- to estimate linkage disequilibrium between SNPs and estimate LD blocks,

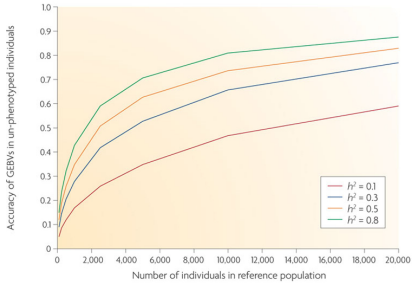
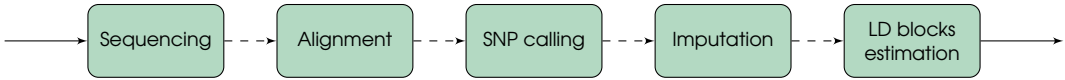
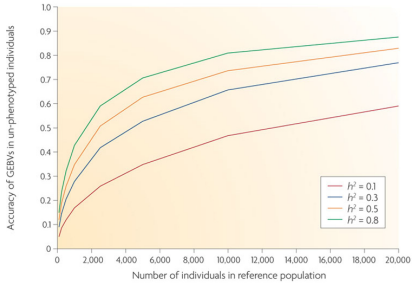


Fig. Accuracy of GEBVs of un-phenotyped individuals (Goddard and Hayes, 2009).

Methods, WGS



- individual WGS,
- 126 bulls with high and low EBVs for longevity,
- mean coverage 1.5x per individual bull,
- to estimate linkage disequilibrium between SNPs and estimate LD blocks,
- the lack of power...



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Results, Pathway analysis

Fig. Overall representation of Gene Ontology terms in more than 1400 cattle genes.

- selection for RL is selection for Fertility, Longevity, Ability to combine reproduction and lactation till late in life, Health and Robustness

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Fig. Overall representation of Gene Ontology terms in more than 1400 cattle genes.

- selection for RL is selection for Fertility, Longevity, Ability to combine reproduction and lactation till late in life, Health and Robustness
- results of pathway analysis support the above