The effect of host genetics factors on shaping pig gut microbiota

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Introduction

- Next Generation Sequencing methods enable to characterize the whole microbiome of an ecosystem

- The gut microbiome plays a major role in the immune system development, state of health and energy supply to the host

- Many factors influence the microbial composition in the gut:
  - feeding
  - housing
  - age
  - host genetic background

Introduction

- The host genetics can influence the microbiota composition due to
  - differences in immunoglobulin and antibacterial molecules secreted into the gut lumen (Wen *et al.*, 2008)
  - differences in the mucosal gut structure (Sommer et al., 2014)
  - differences in bile acid metabolism (Ryan et al., 2014)

- In pig production systems growth performance is from great interest
  - ’ investigating the influence of the microbial community in the gut on growth performance

Aim of the study:

- Describing the bacterial gut microbiome of purebred Piétrain sows
- Estimating genetic parameters of the microbiota composition in the porcine gut (heritabilities of microbial abundances and genetic correlations)
- Predicting the phenotype (daily gain and feed conversion) from the microbial community (microbial prediction)

Methodology

207 Piétrain sows

Colon digesta samples

DNA extraction

16S Illumina-amplicon sequencing

Blood samples

DNA extraction

Illumina 60k genotyping

Bioinformatic and statistical analysis

Describing bacterial community

Genomic relationship matrix

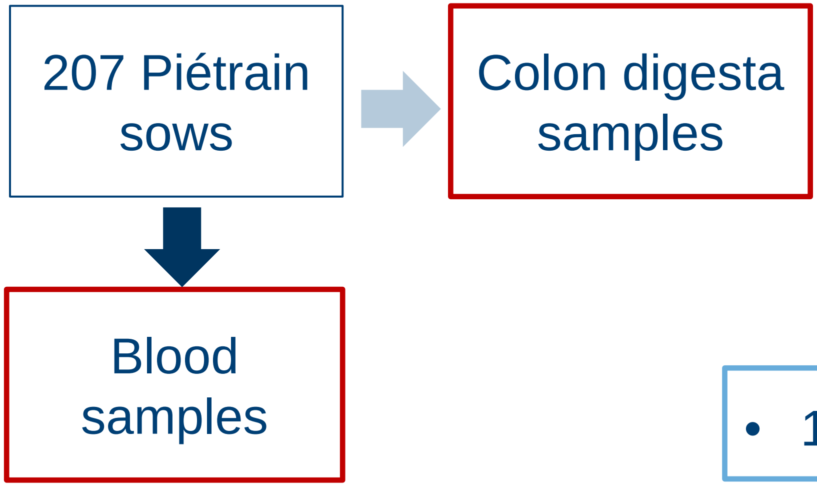
Estimating genetic parameters
Microbial prediction

Methodology

207 Piétrain sows

- Standardized feeding and housing at LSZ Boxberg (ZDS, 2007)
- Performance testing from 30kg-105kg of daily gain (DG) and feed conversion(FC)

Methodology



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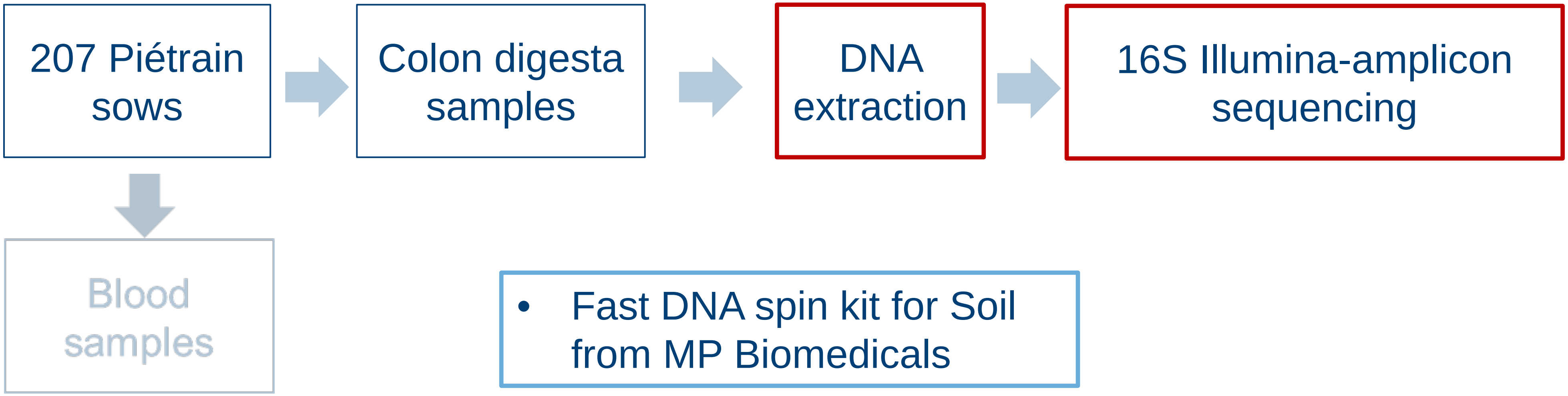
Colon digesta samples

Blood samples

- 14 Slaughter days

Methodology



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Colon digesta samples

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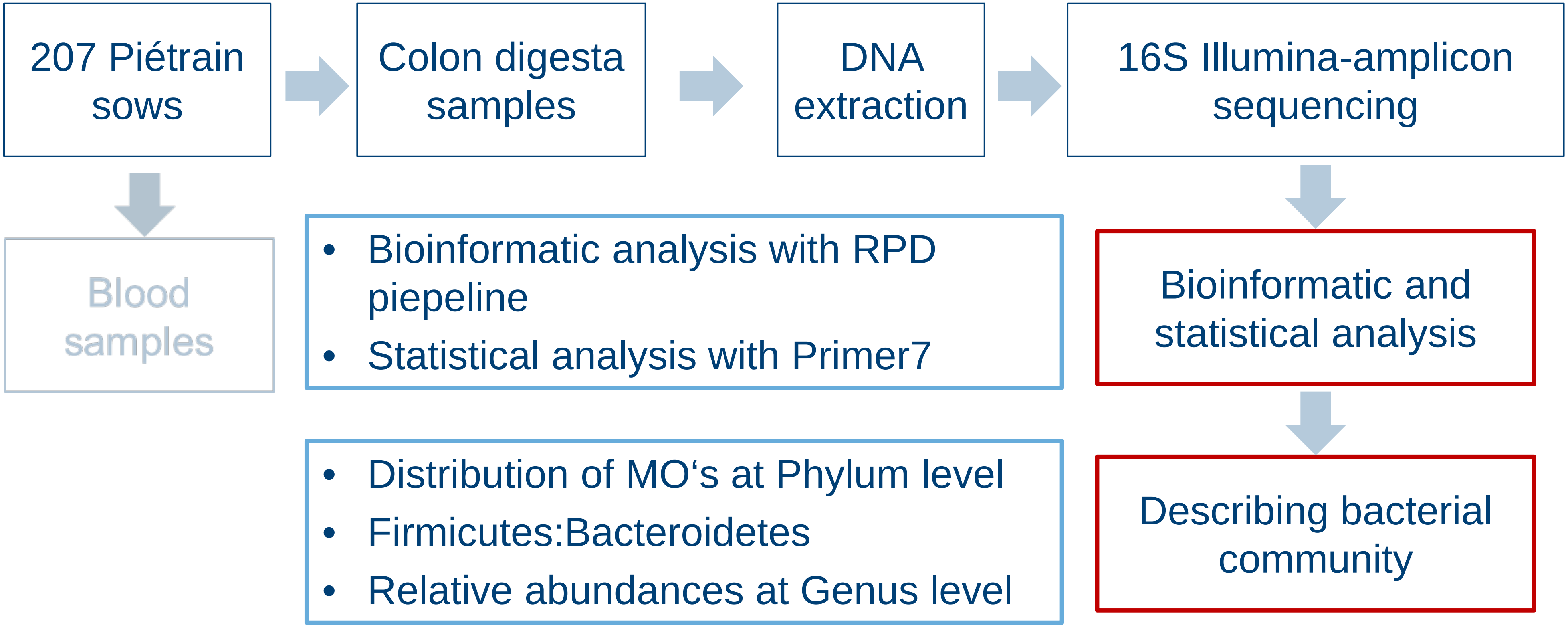
Blood samples

- Fast DNA spin kit for Soil from MP Biomedicals

- Sequencing of the V1-V2 region of the 16S rRNA gene

Methodology



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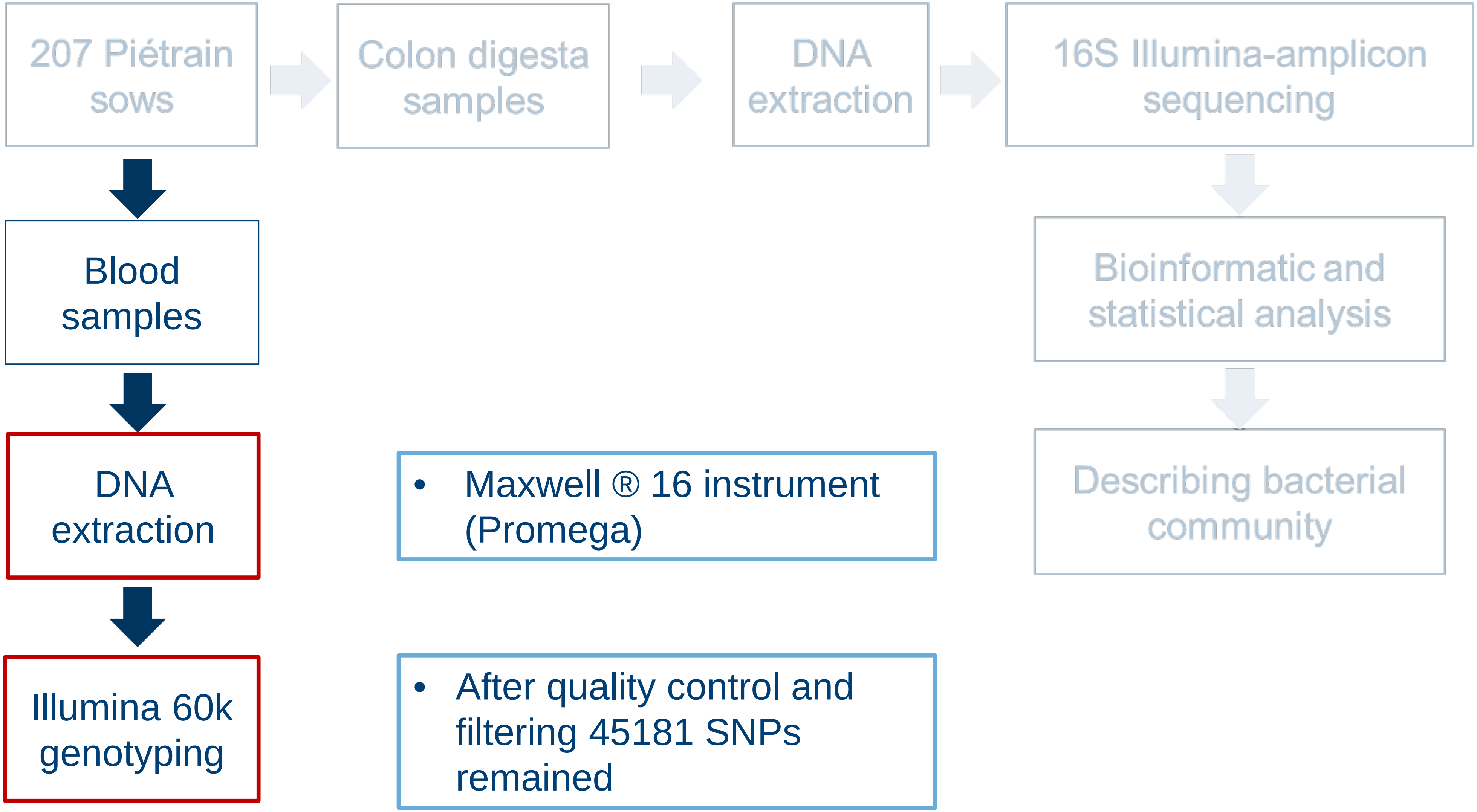
- Bioinformatic analysis with RPD piepeline
- Statistical analysis with Primer7

Bioinformatic and statistical analysis

- Distribution of MO's at Phylum level
- Firmicutes:Bacteroidetes
- Relative abundances at Genus level

Describing bacterial community

Methodology



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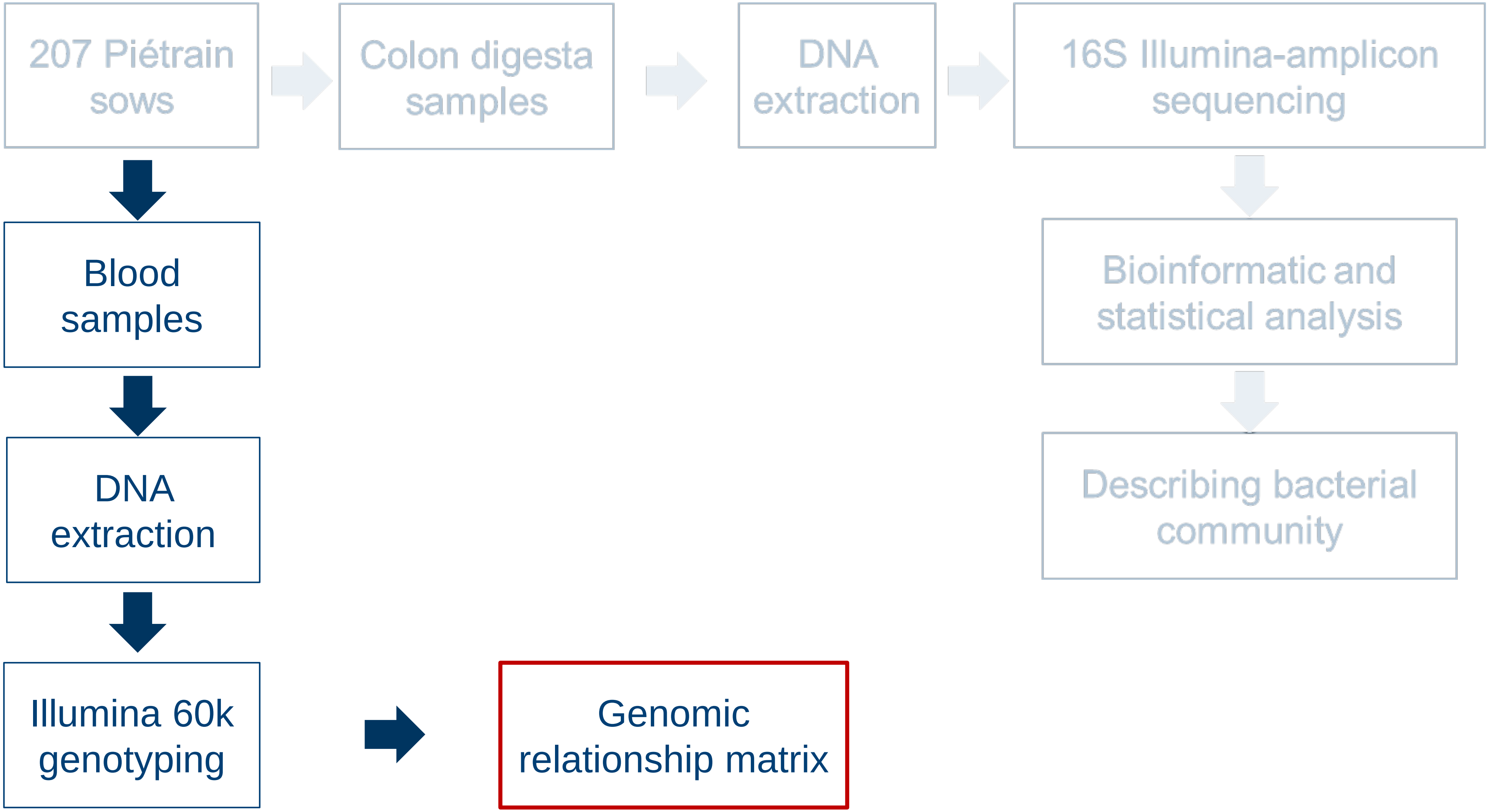
DNA extraction

- Maxwell ® 16 instrument (Promega)

Illumina 60k genotyping

- After quality control and filtering 45181 SNPs remained

Methodology



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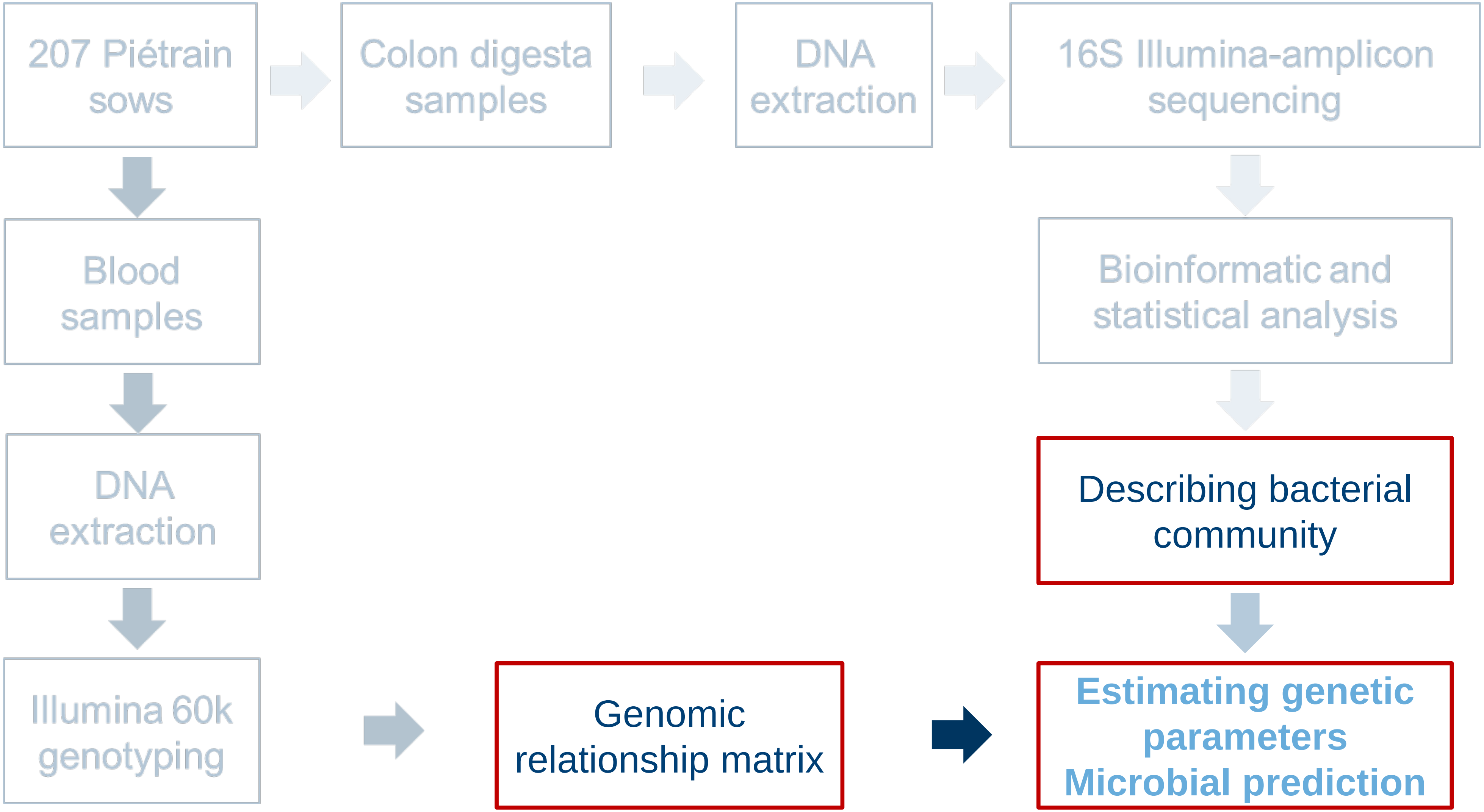
Illumina 60k genotyping

Genomic relationship matrix

Methodology



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Genomic relationship matrix

Estimating genetic parameters
Microbial prediction

Statistics – genetic parameters

- Univariate and pairwise bivariate analysis with ASReml in R for each bacterial Genus (51 Genera with a relative abundance >0.1)
- Fixed effects were estimated separately by fitting a linear mixed model to each Genus
- Genomic mixed linear models were used to estimate genetic parameters:

$$y = Xb + Z_{SD}SD + Z_a a + e$$

y	Vector of observations (relative abundances of bacterial Genera)
b	Vector of fixed effects
SD	Vector with random slaughter day effects
a	Vector with random additive-genetic effects of the animal
$X, Z_{SD,}\, Z_a$	Corresponding design matrices
e	Residual term

Statistics – genetic parameters

- The covariance structure of the random animal effect was

$$var(a) = G * \sigma_a^2$$

G genomic relationship Matrix (VanRaden, 2008)
σ_a^2 additive genetic variance

- P-values of heritabilities were estimated by performing a Likelihood-Ratio test of the random animal effect

Statistics – microbial prediction

- Prediction of the phenotype (DG, FC) based on bacteria at OTU level
- Less abundant genera were removed from the dataset
- After log transformation the data were standardized to a mean of 0 and a standard deviation of 1
- Calculating the microbial relationship matrix M:

$$M = XX'/m$$

X	$n * m\ Matrix$
n	Samples
m	OTUs

Statistics – microbial prediction

- Fitting a G-BLUP model to predict the phenotype with the package rrBLUP in R:

$$y = Xb + Zg + e$$

y	Vector of observations (DG,FC)
b	Vector of fixed effects
X	Corresponding design matrix
g	Random effect of the OTUs, with $g \sim N(0, M\sigma_g^2)$
Z	Design matrix containing the individual's relative abundances of the OTUs
e	Residual term

Statistics – microbial prediction

- Five way cross validation was performed
  - Splitting the data in 5 equally sized groups
  - Predicting each group from the other four groups
- Accuracy of prediction was determined by Pearson’s r (correlation between observed and predicted phenotype)

Results: Microbial community

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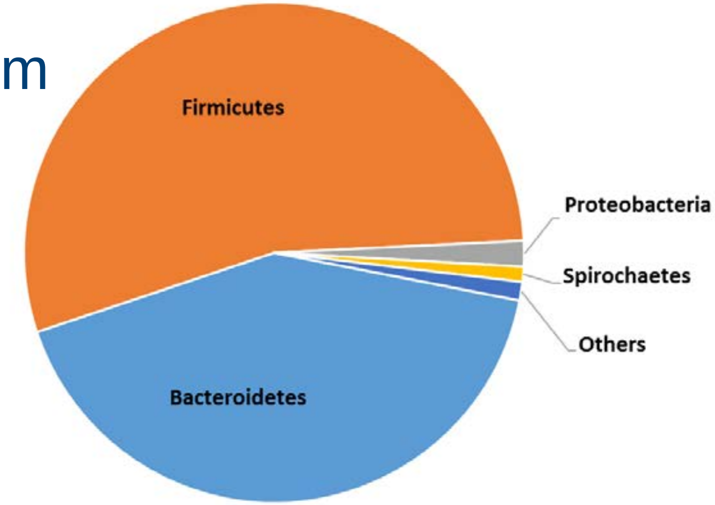
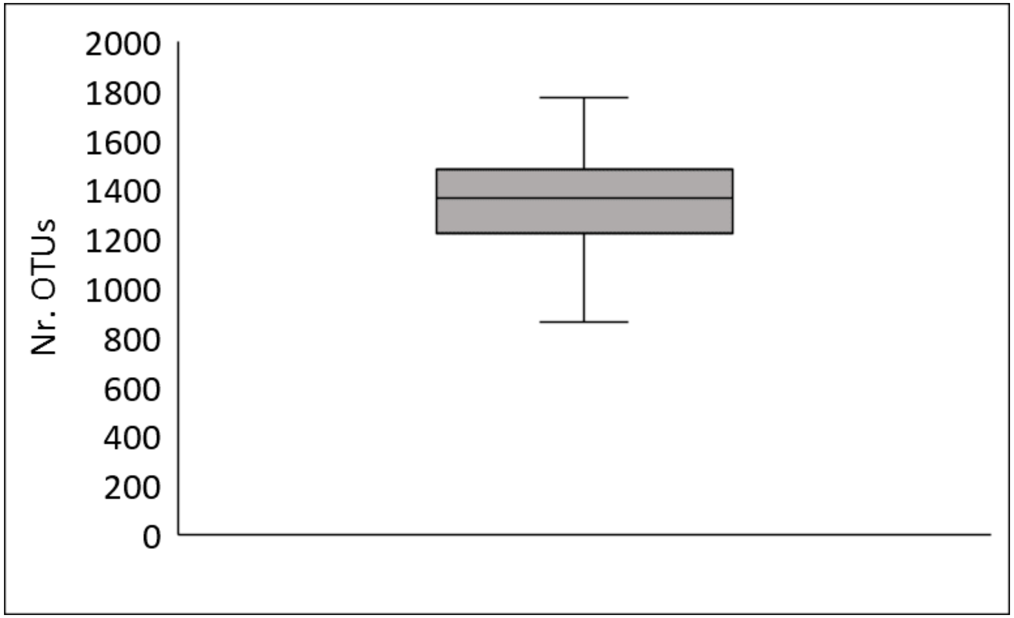
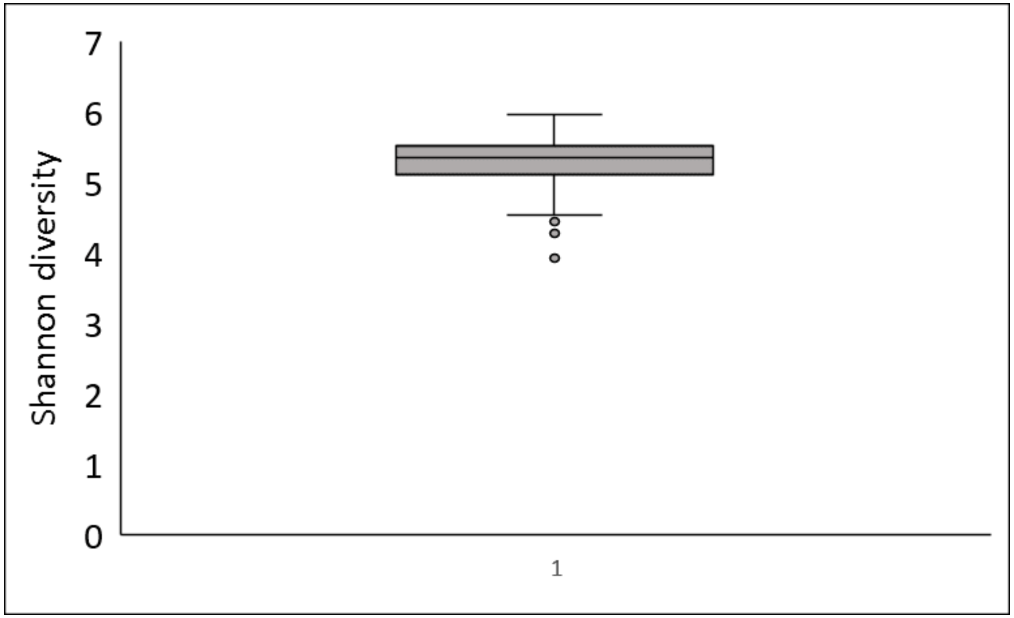
Phylum

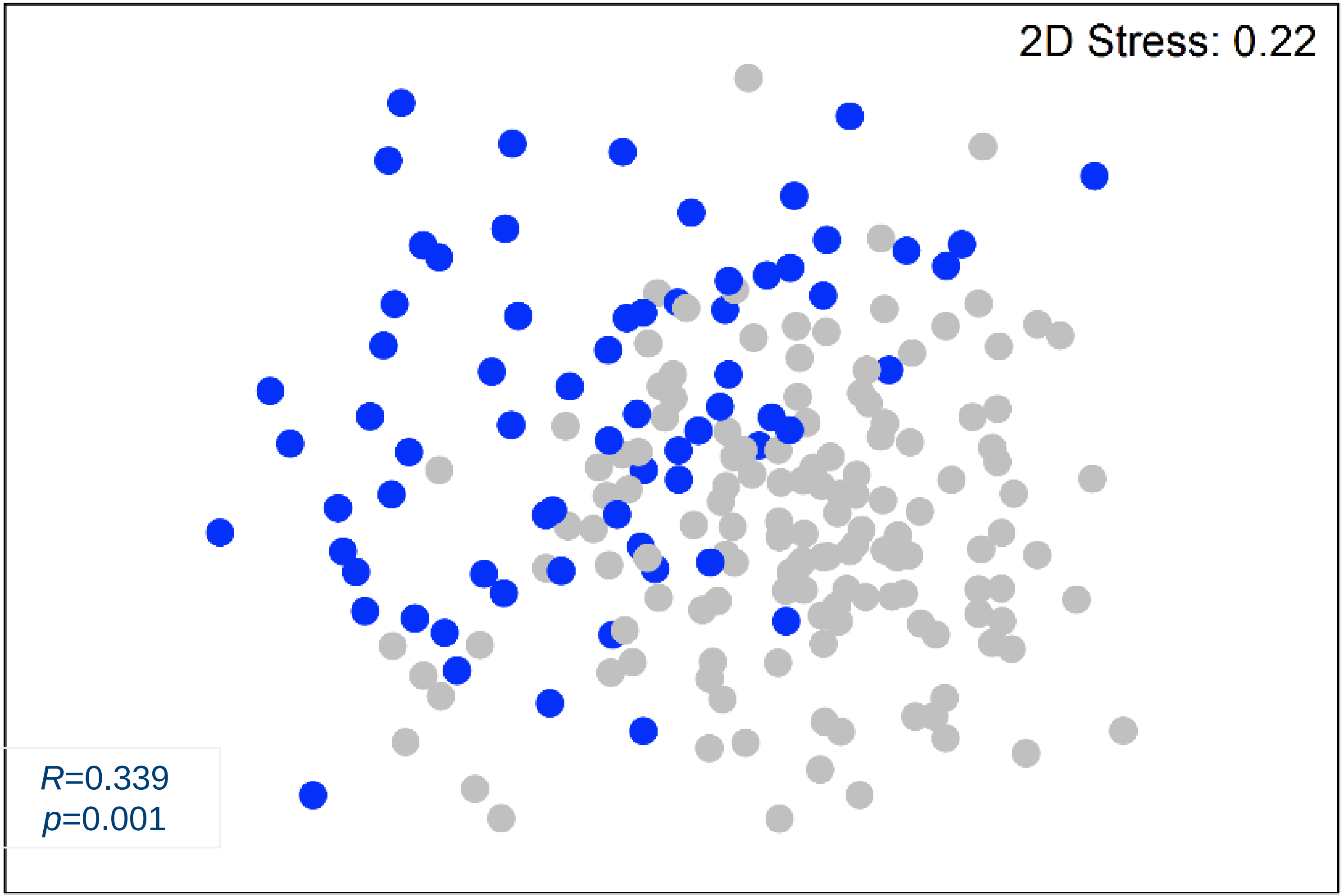


Bacteroidetes	42%
Firmicutes	54%
Proteobacteria	2%
Spirochaetes	1%
Others	1%



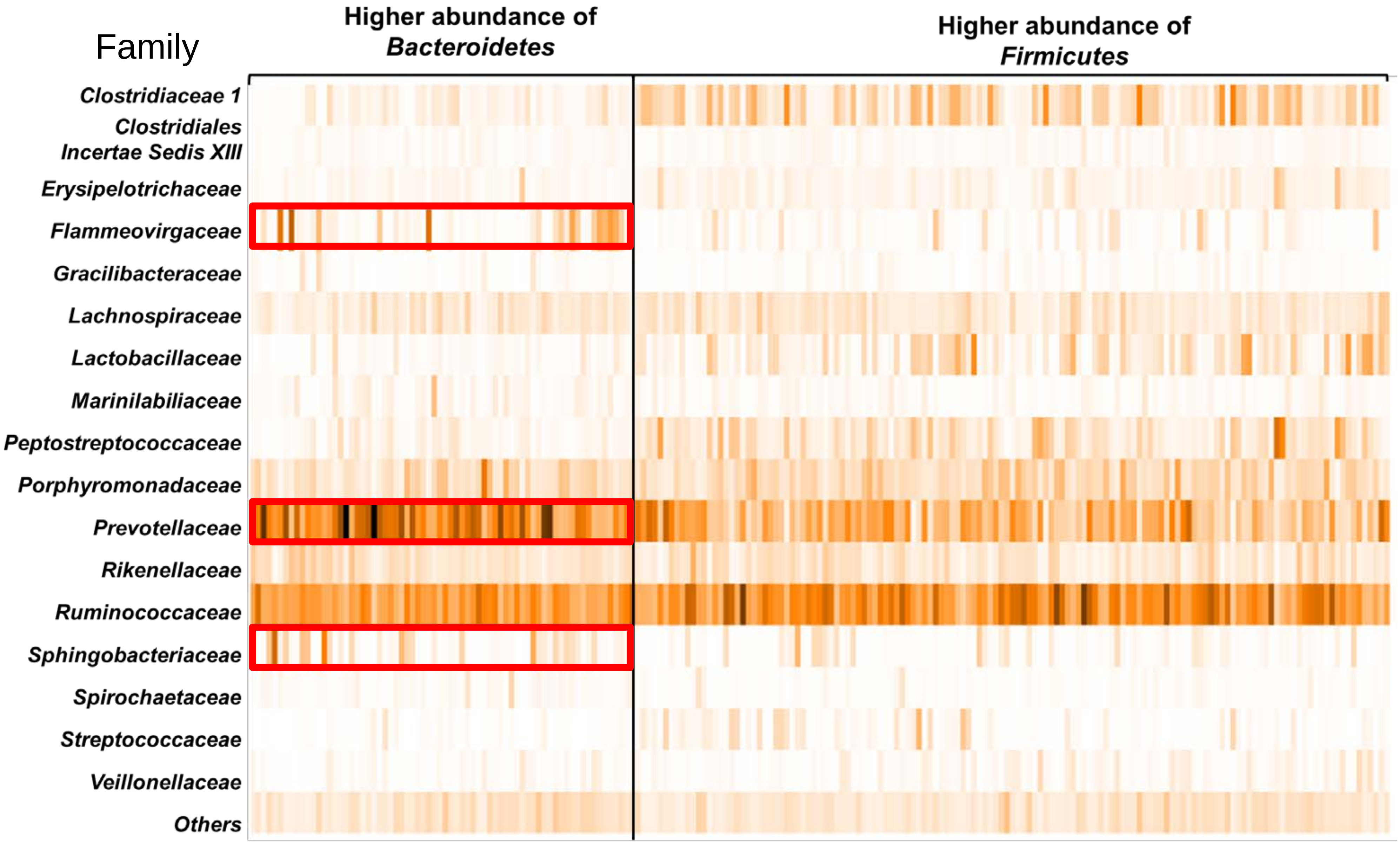


Results: Microbial community

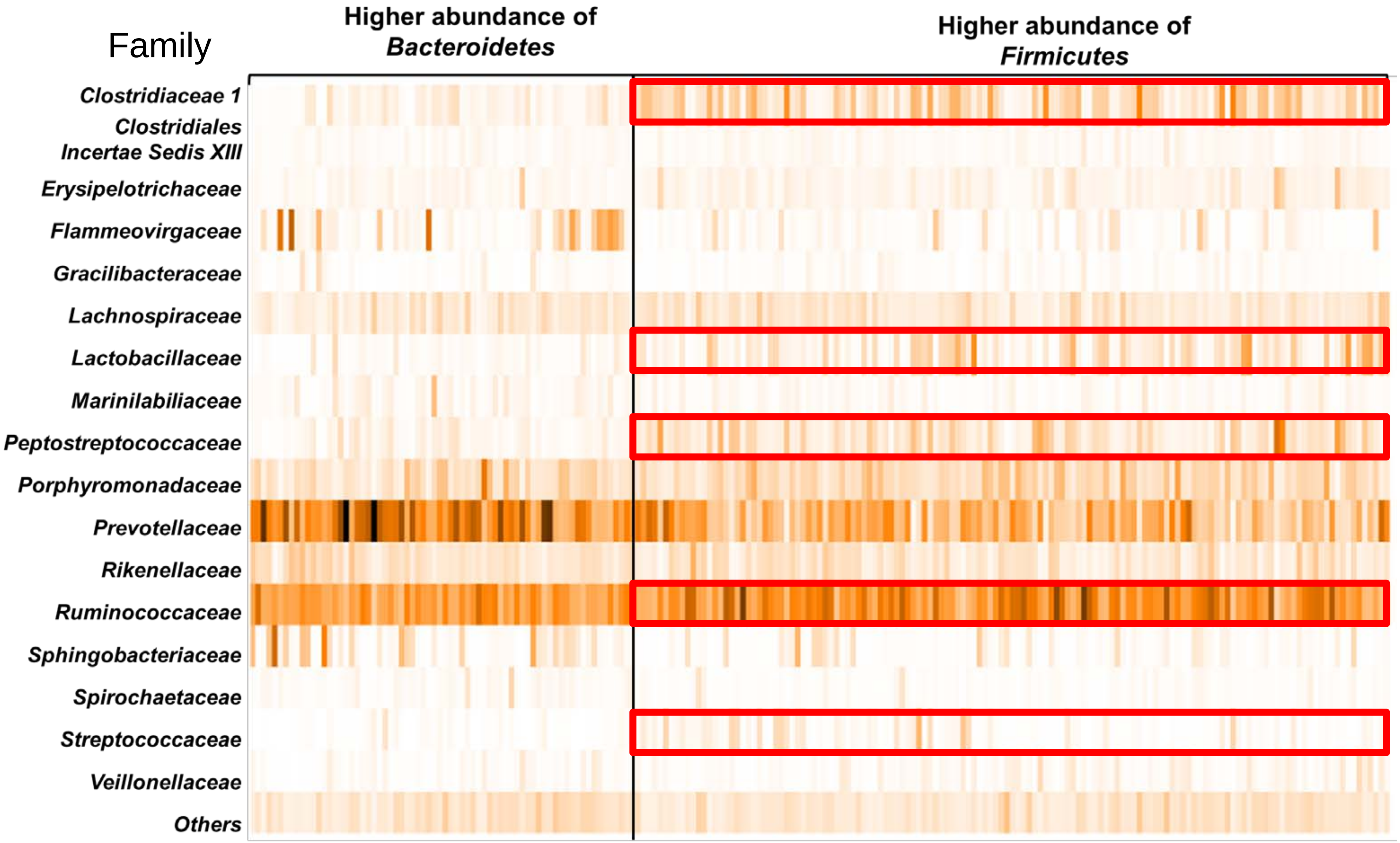


R=0.339
p=0.001

Results: Microbial community



Results: Microbial community



Results: Genetic Parameter

Table: Estimated heritability (h^2), standard error (SE) and p-value for the relative abundances of bacterial genera

Bacteria	h^2	SE	p-value
Alloprevotella	0.36	0.09	0.01
Blautia	0.36	0.10	0.00
Catenibacterium	0.39	0.00	0.01
Lactobacillus	0.34	0.15	0.03
Unc. Firmicutes	0.28	0.11	0.02
Unc. Proteobacteria	0.29	0.00	0.01
Unc. Spirochaetales	0.52	0.05	0.00
Unc. Spirochaetes	0.32	0.00	0.01
Unc. Succinivibrionaceae	0.57	0.04	0.00
Unc. Veillonellaceae	0.32	0.14	0.01

Based on 51 bacterial Genera 10 showed significant heritabilties with a p-value < 0.05

Results: Microbial prediction

- To link the phenotype with the microbial community a microbial prediction was performed
- This is similar to genomic prediction, but instead of using the SNP data as explaining variable microbiota data were included
- The five way cross validation resulted in a prediction accuracy of 0.39 for daily gain and 0.10 for feed conversion

Conclusion

- The gut microbiota in Piétrain pigs is influenced by the genetics of the host
- Based on microbial prediction the influence of the gut microbiota on growth performance has been shown

These results show various possibilities, e.g. concerning biological explanations and an improved nutrient supply caused by breeding

Thank you for your attention!

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