



Systems biology and RNA-Seq analyses to detect biomarkers for metabolic syndrome in a porcine model

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Introduction

Metabolic syndrome (MS):

- not a disease
- clustering of risk factors
 - Excess body weight
 - High blood pressure
 - High insulin levels
 - Dyslipidemia



MS responsible
for:

Cardiovascular disease morbidity
Type 2 diabetes

Introduction

Studies of metabolic syndrome - past and future

- In the past

Genome Wide Association Studies (GWAS)

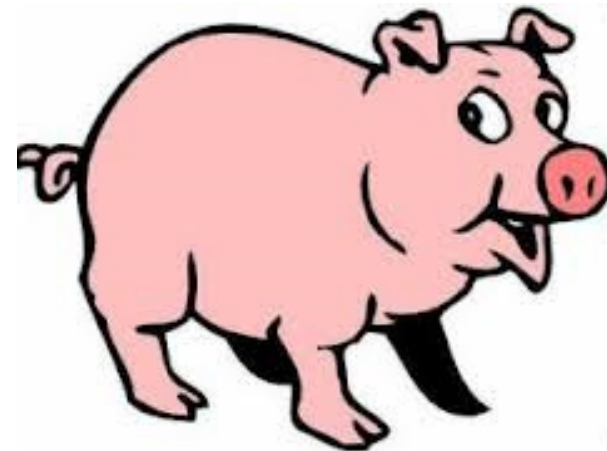
- Future approaches
 - Genome wide expression data with high throughput technologies
 - Proteomics
 - Bioinformatics to combine large datasets



Introduction

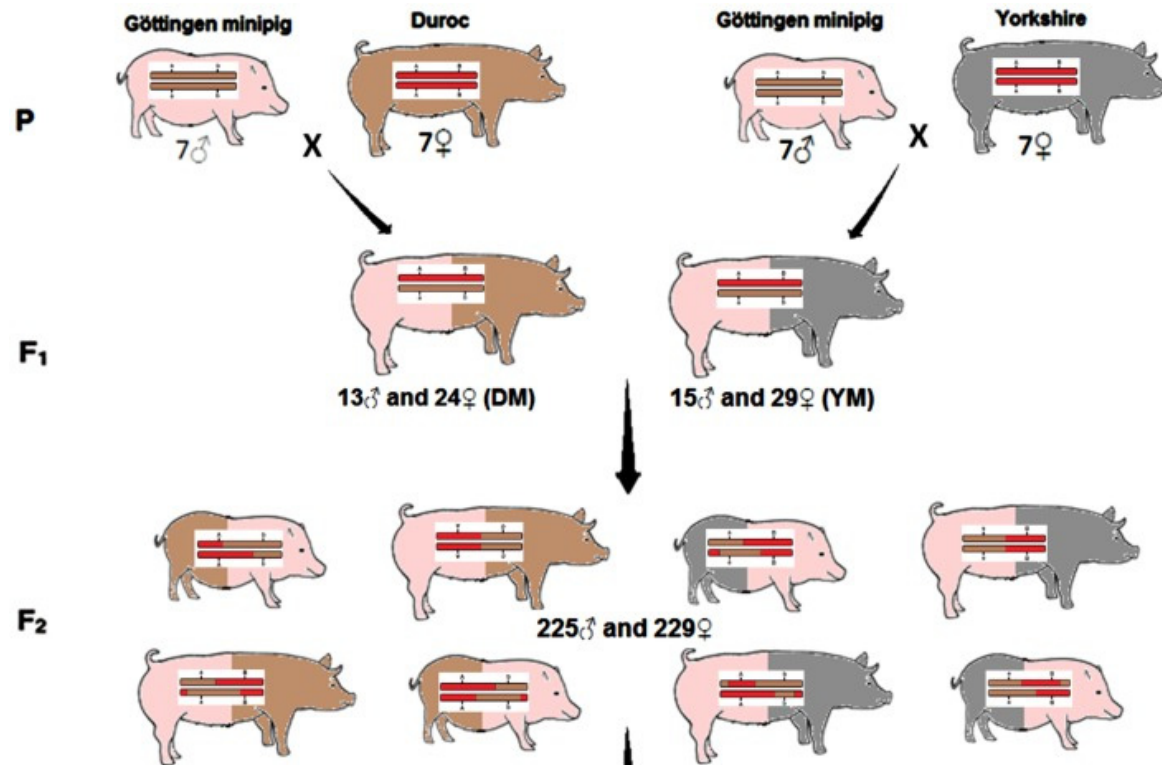
Dissect the genetic factors which are common among the different metabolic disorders of MS and detect biomarkers.

The project is unique because ...
the first implementation of a network based analysis in large sample of pigs.



Dataset

Experimental design



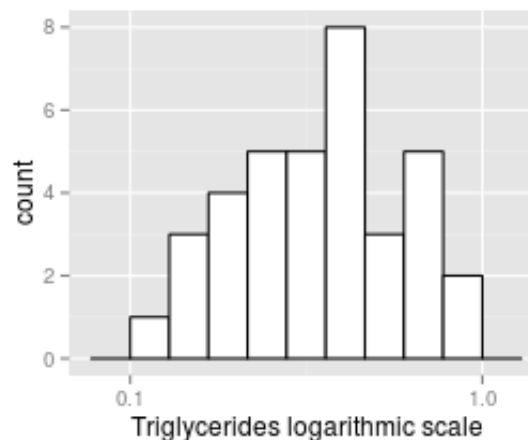
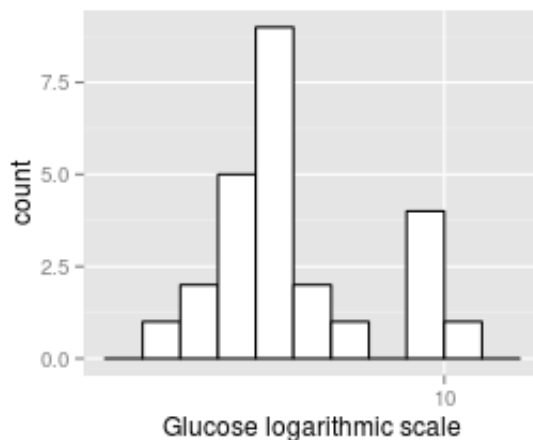
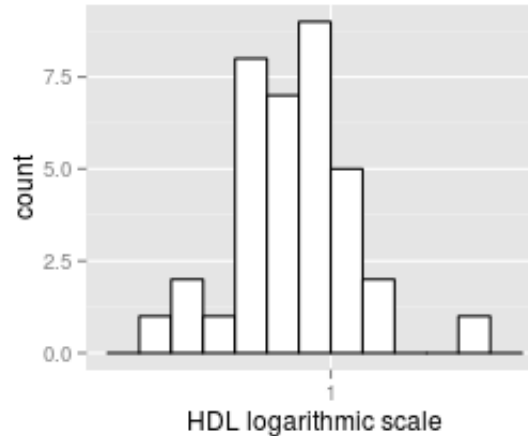
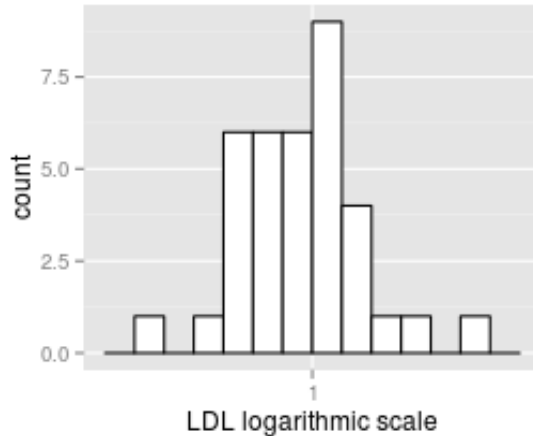
source: LJA Kogelman et. al Front. Genet. 2013

An F2 resource population for obesity and obesity related studies.

- Phenotyped for several obesity and obesity related traits.
- RNA-seq of subcutaneous adipose tissue for 36 animals

Methods and Results

Distribution of the phenotypic measurements



- Experimental groups were designed based on the 25th percentile of the samples with the lowest and the highest values.

Methods and Results

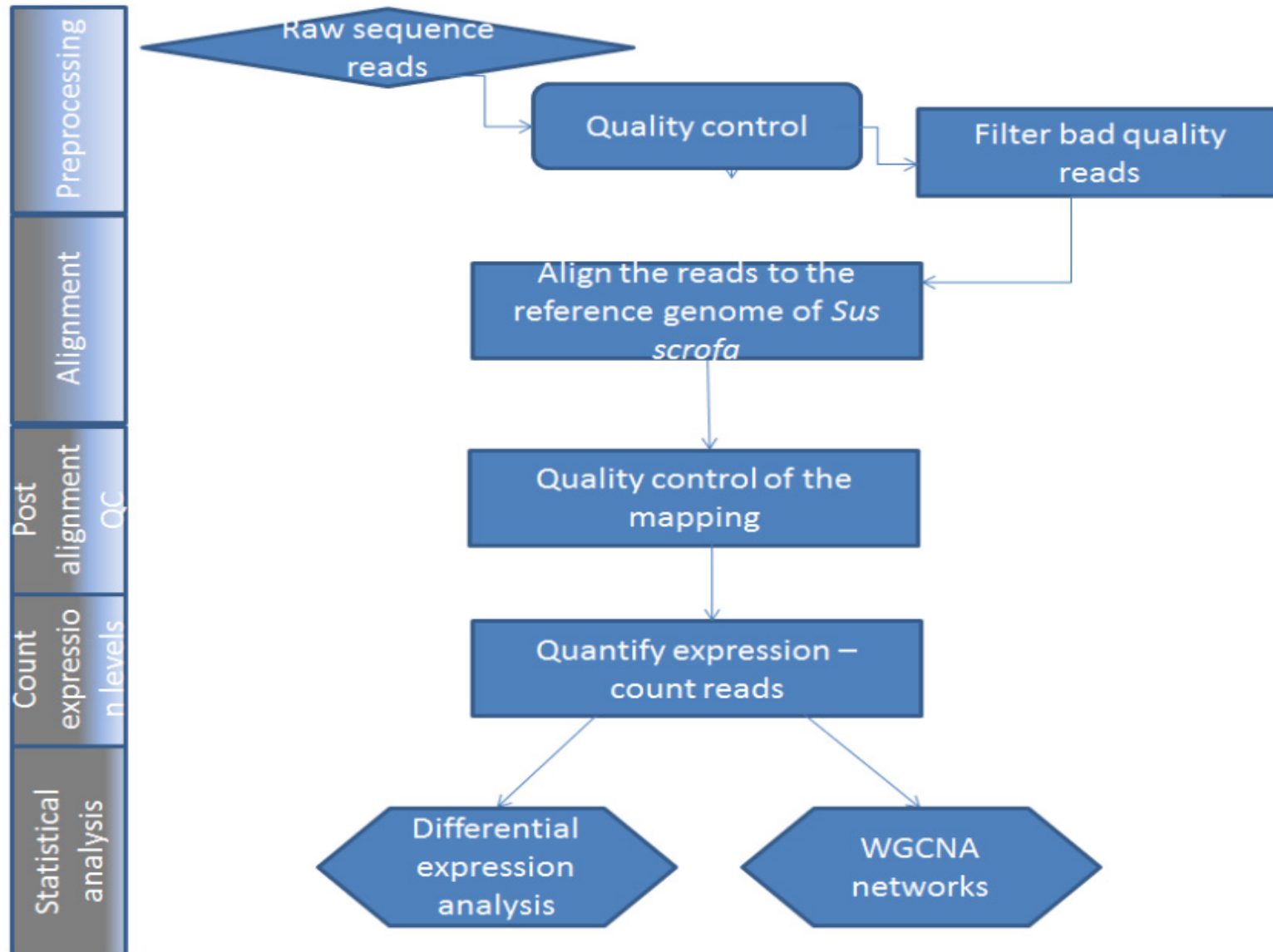
RNA sequencing data

- Illumina – HiSeq-2500 high-throughput sequencing machine
- 9 samples per lane
- Paired-end technology
- 100bp reads



Methods and Results

RNA-seq workflow – from reads to counts

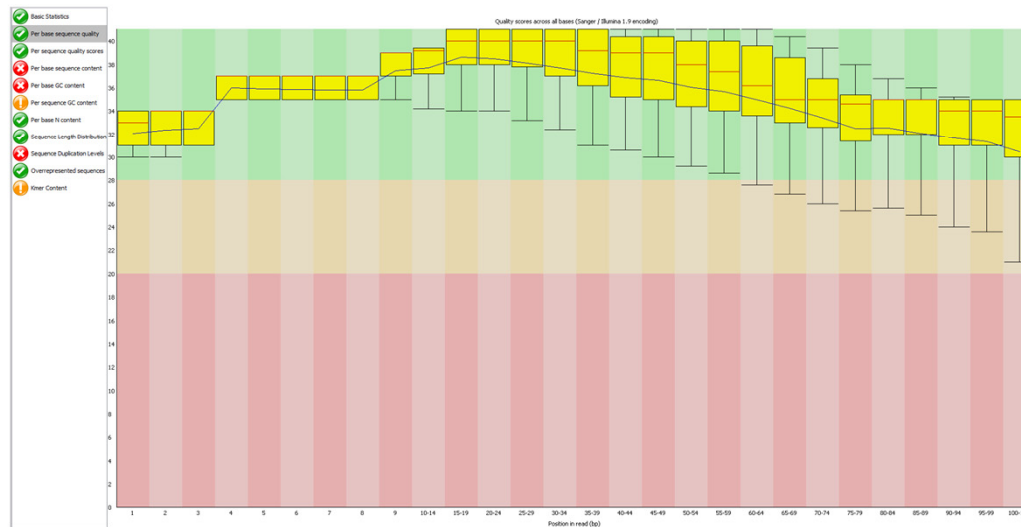


Methods and Results

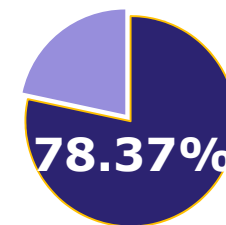
RNA-seq statistics

RNA-Seq S/C Fat: pre-QC using FAsTQC

Quality scores of the reads and filtering



Mapping Stats



■ MAPQ \geq 30

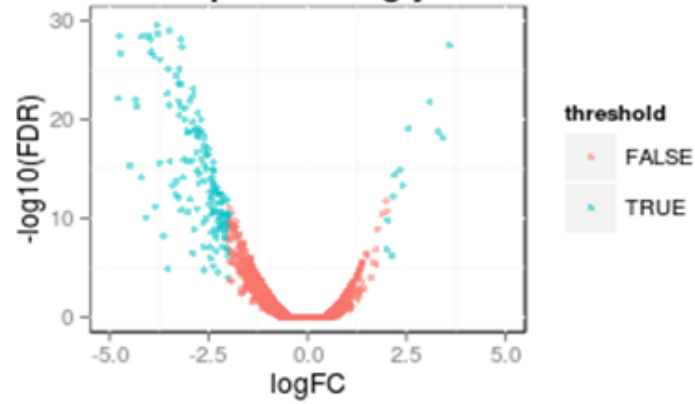
In average : 36 m. reads/library



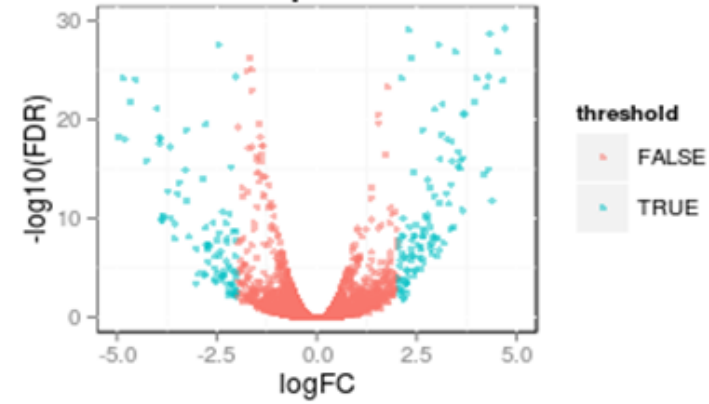
Methods and Results

Differential expression (DE) analysis

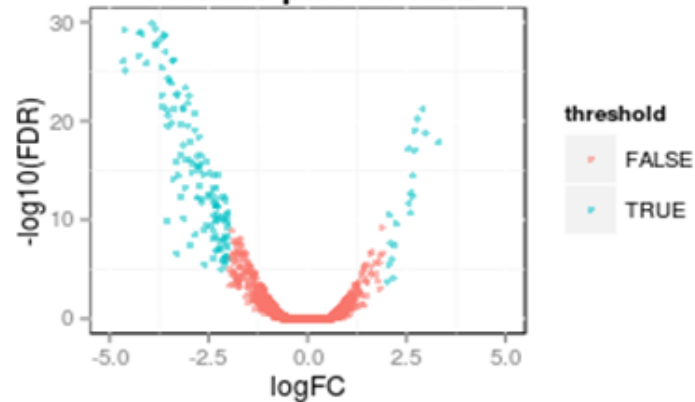
Volcano plot for Triglycerides



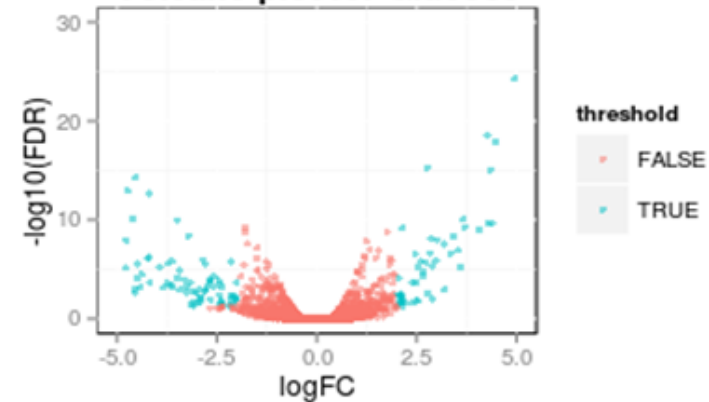
Volcano plot for LDL



Volcano plot for HDL



Volcano plot for Glucose



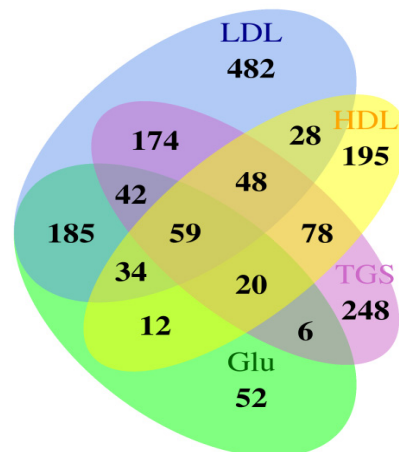
Methods and Results

Differential expression (DE) analysis

Enrichment analysis of DE genes shows genes involved to homeostasis and inflammation

59 DE genes shared in all experimental groups.

Functional enrichment shows genes are associated with disorders related to metabolic syndrome



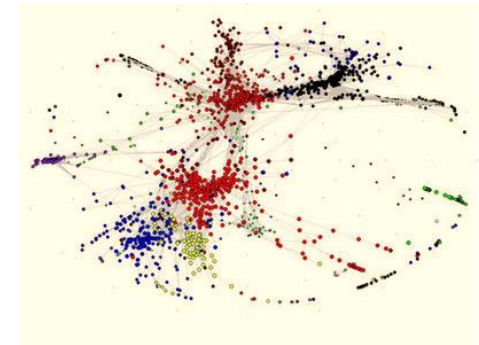
Methods and Results

Weighted Gene co-expression networks - WGCNA

Study of highly interconnected genes ("modules") which are likely involved in the same pathway.

Metric of pairwise relationship of the genes : correlation

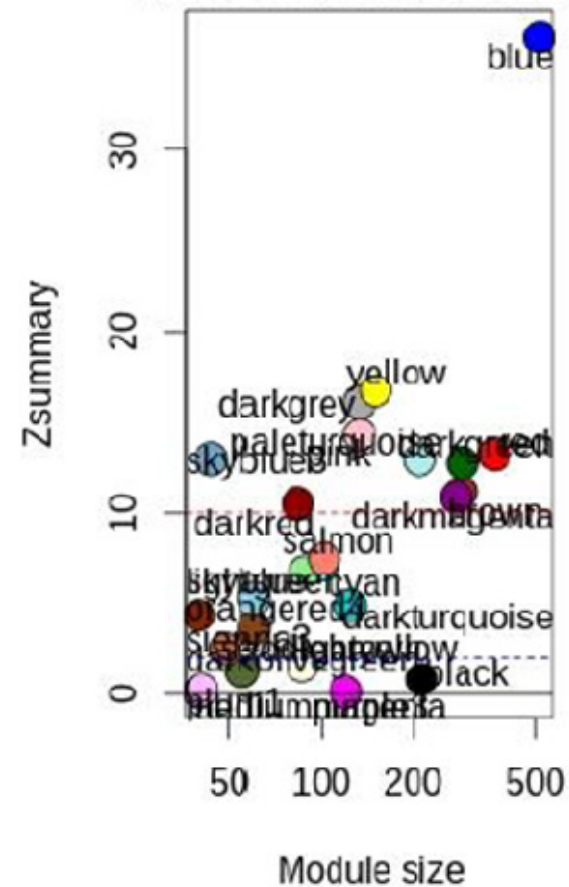
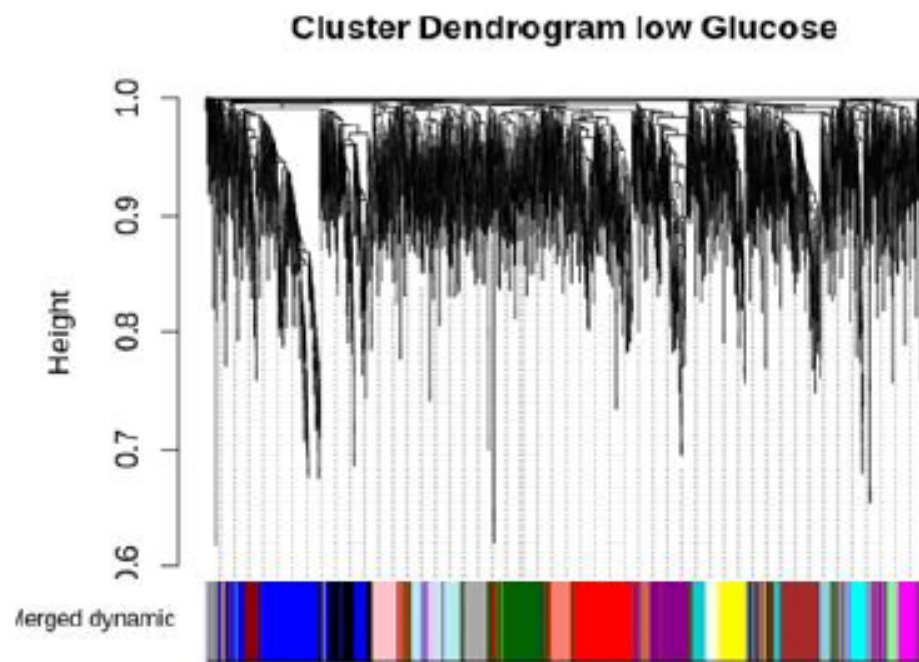
Comparison of "modules" among the experimental groups. Detection of perturbed "modules".



Methods and Results

Module preservation statistics
for glucose group

Define modules



Discussion – Future perspectives

- Indeed shared DE genes among experimental groups
- Over-represented GO terms related to metabolic disorders.
- More phenotypes to be analyzed
- Differential co expression analysis



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