Whole liver transcriptome analysis for the metabolic adaptation of dairy cows

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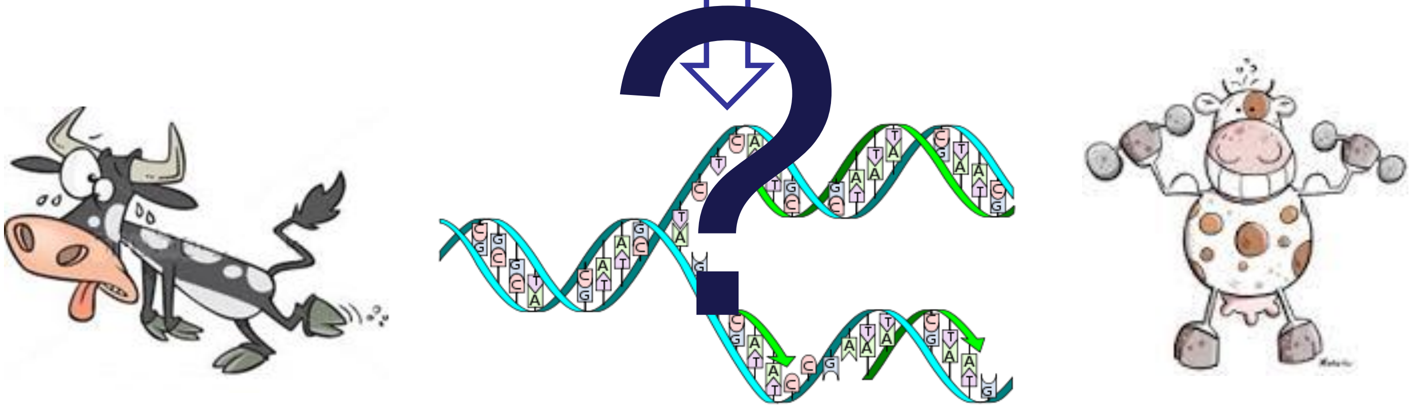
Motivation – ***Background***

- Selective breeding of high-yielding dairy cows
  - ⇒ up to 60 kg milk per day
- High energy demand can not be fully covered by food intake
  - ⇒ negative energy balance during their early lactation
- Mobilization of body fat, protein and mineral stores
  - ⇒ adaptation of the hepatic metabolism

Motivation – *Metabolic Adaptation*

Why does the success of adaptation differ substantially between cows - even under the same conditions and similar production levels?

Ingvartsen et al. (2003)
Drackley et al. (2005)
Graber et al. (2010)



Project OnlyRobust – *Overview*

Goal: Identification of candidate genes and pathways important for the metabolic adaptation

Study 1: Genomic Level:

„Gene-based mapping and pathway analysis of metabolic traits in dairy cows“ (Ha et al., 2015)

Study 2: Transcriptomic Level:

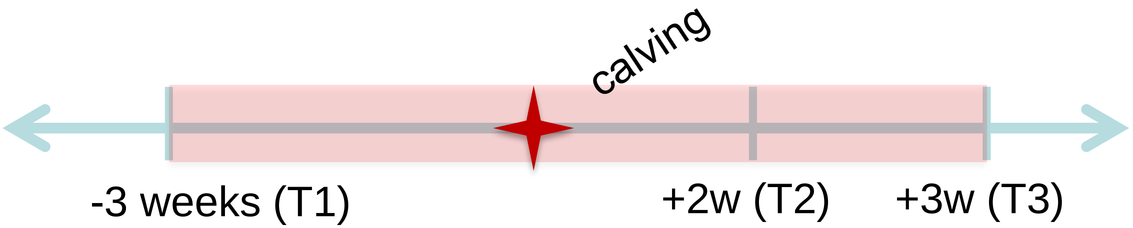
„Whole liver transcriptome analysis for the metabolic adaptation of dairy cows“ (in preparation)

Data – ***Experiment Design***

Identification of genes und pathways, important for metabolic adaptation during the transition period

Data:

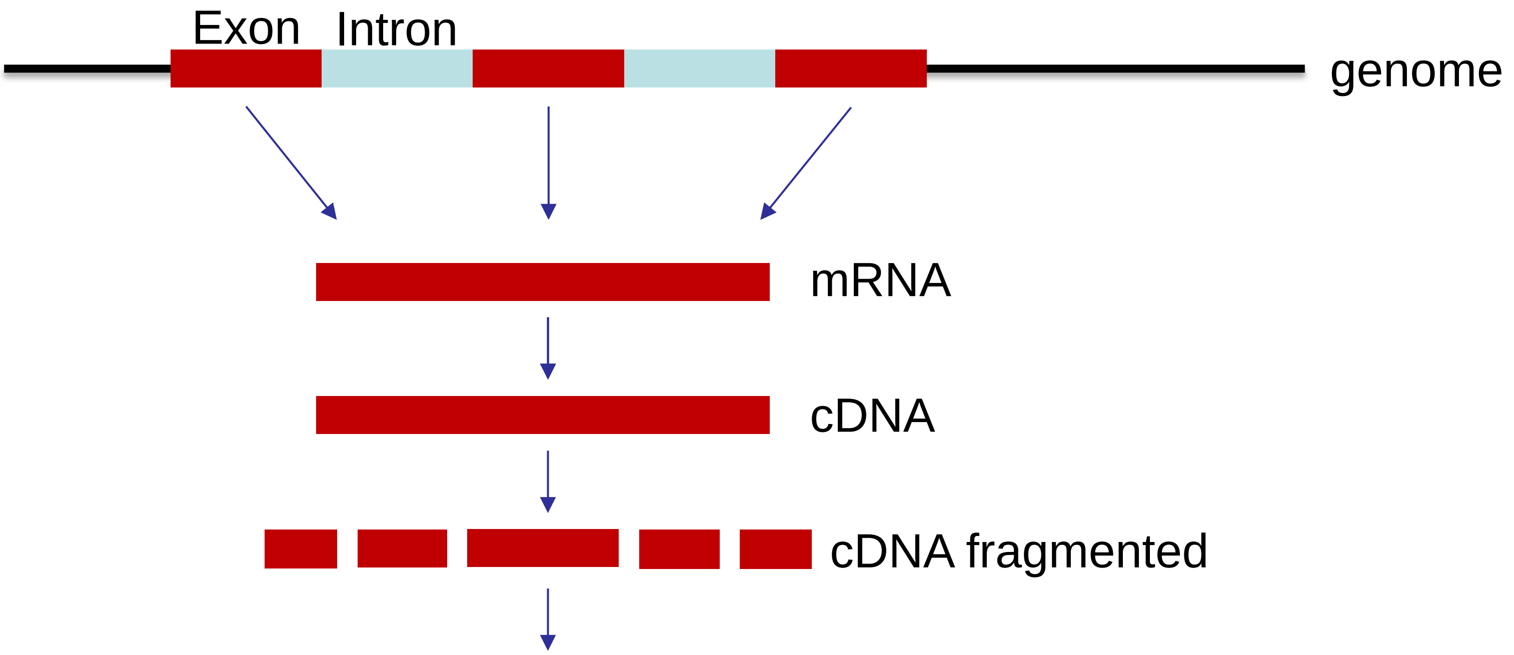
- *Liver samples of 6 dairy cows at different points of time:*



- *RNA-Sequencing*

Differential Gene Expression and Pathway Analysis:
T1 vs. T2
T1 vs. T3
T2 vs. T3

Data – *RNA-Seq*



Exon Intron

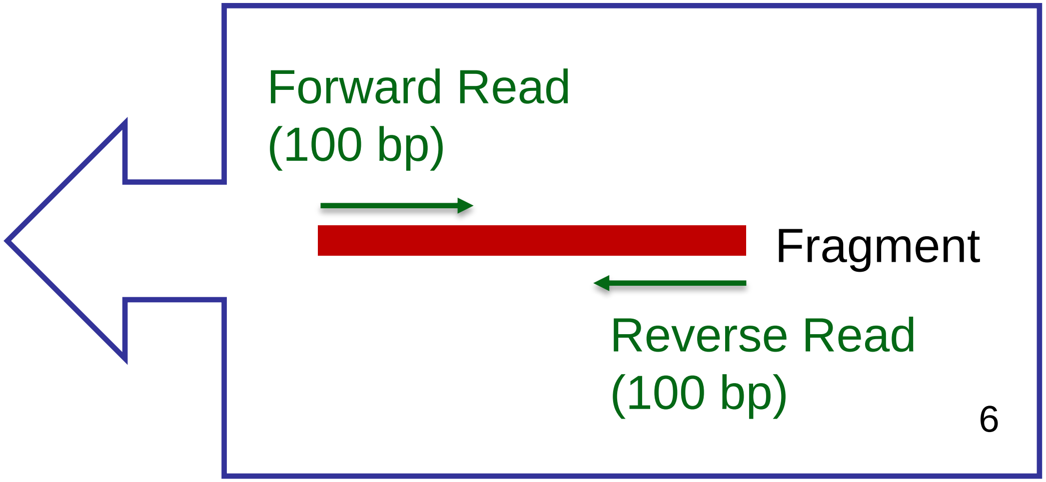
genome

mRNA

cDNA

cDNA fragmented

Illumina HiSeq 2000

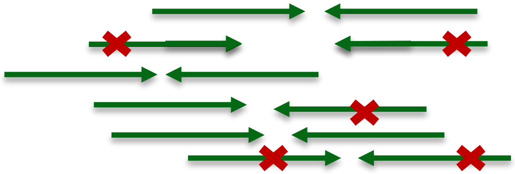


Forward Read
(100 bp)

Fragment

Reverse Read
(100 bp)

RNA-Seq – Pipeline



(Trimmomatic V0.33, Bolger et al., 2013)

(STAR V2.4,
Dobin et al., 2013)

Bos Taurus
(UMD 3.1, release 85)

2 Counts

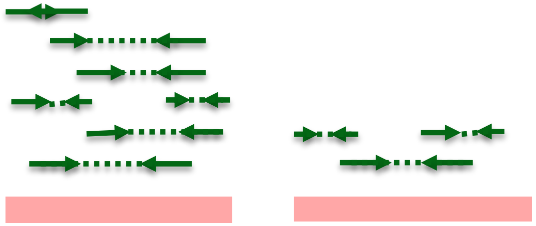
4 Counts

Count-Matrix (after mapping and counting of the reads):

	Sample 1	**Sample 2**	**Sample 3**	**…**
Gene 1	26‘432	20‘422	19‘543	…
Gene 2	0	24	3	…
Gene 3	245	432	332	…
…	…	…	…	…

Analysis of RNA-Seq

Differential Gene Expression Analysis (DGEA)

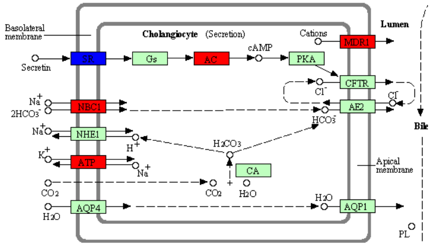


ante-partum

post-partum

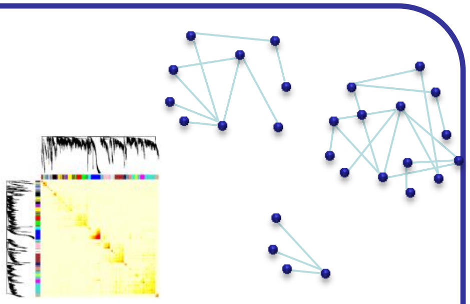
List of differentially expressed genes

Gene Set Enrichment Analysis (GSEA)



KEGG pathways

Weighted Gene Co-expression Network Analysis (WGCNA)



Methods – *DGEA and GSEA*

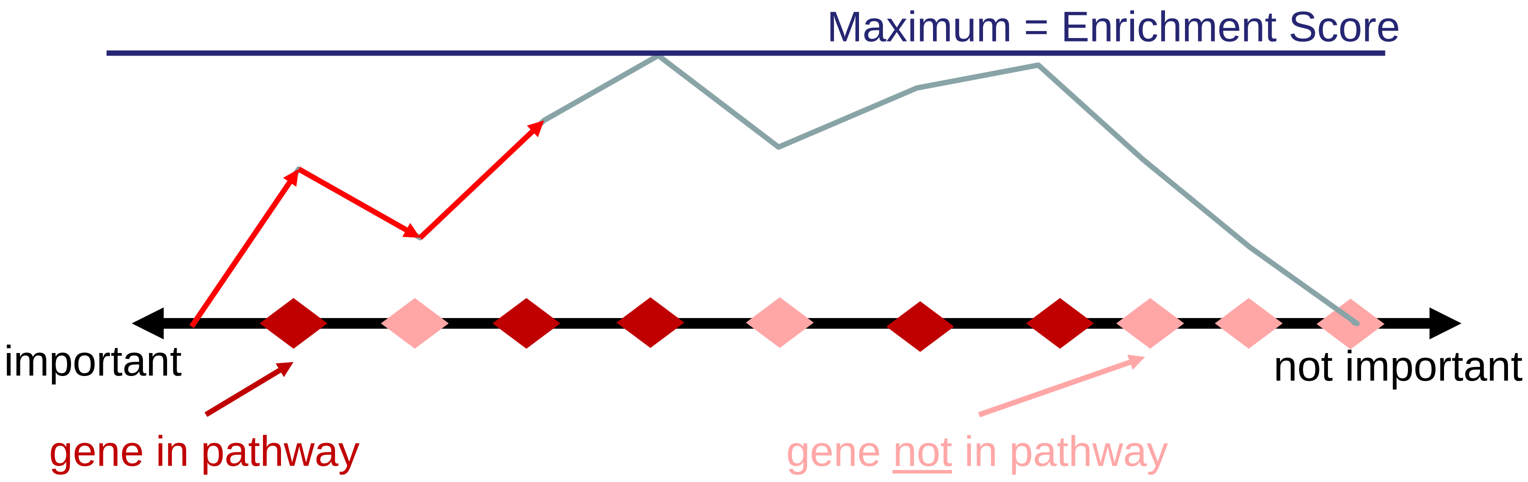
Differential Gene Expression Analysis (DGEA):

- *Generalized Linear Model using negative Binomial distribution („edgeR“, Robinson und Smyth, 2008)*

$$\log(Y) = \mu + \tau_1 \mathrm{cow}_1 + \cdots + \tau_6 \mathrm{cow}_6 + \beta \mathrm{condition} + \log N$$

- *Y = #counts per gene, N = #counts for all genes in a sample*

Gene Set Enrichment Analysis (GSEA, Subramanian et al., 2005):



Maximum = Enrichment Score

important

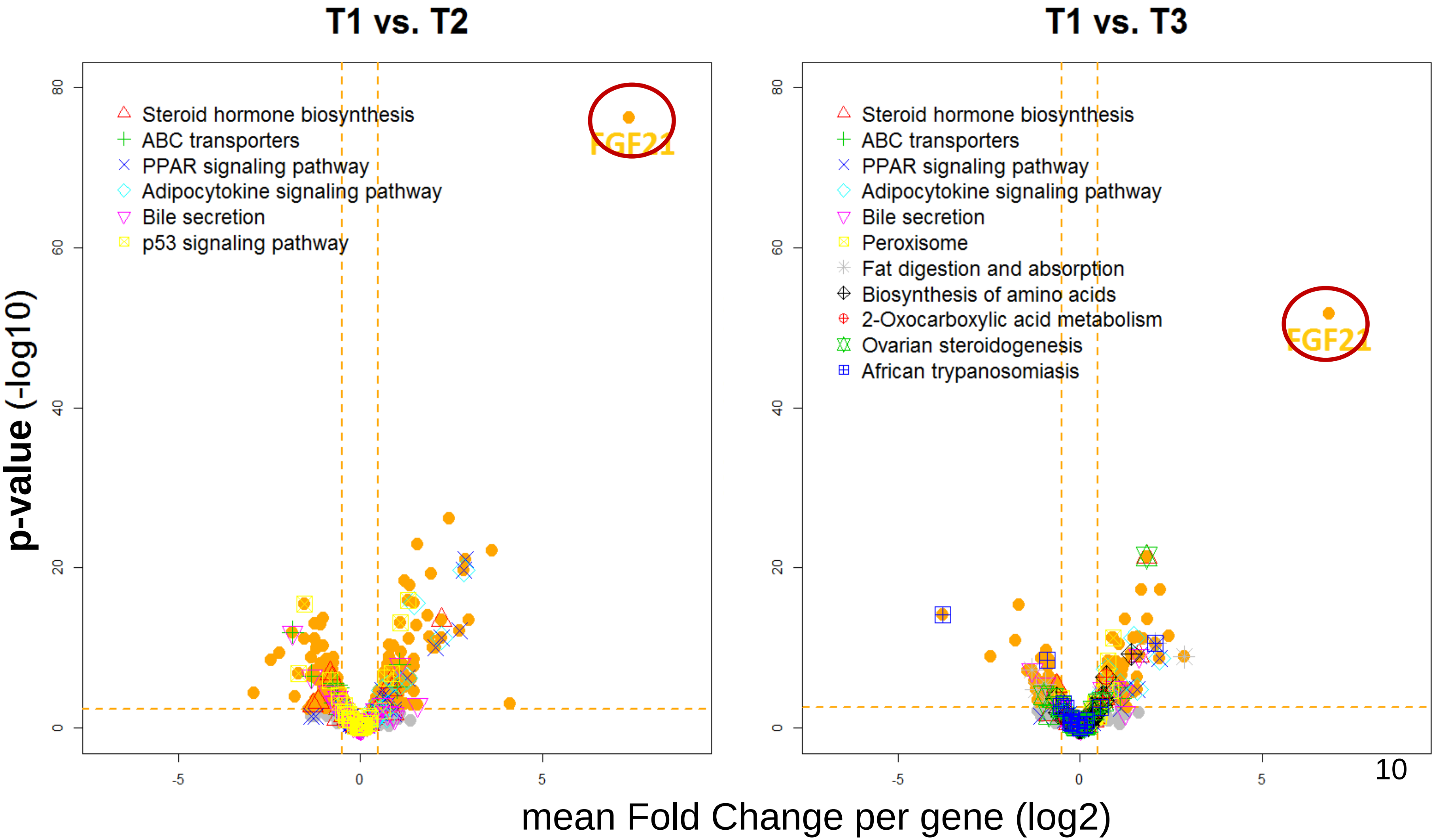
not important

gene in pathway

gene <u>not</u> in pathway

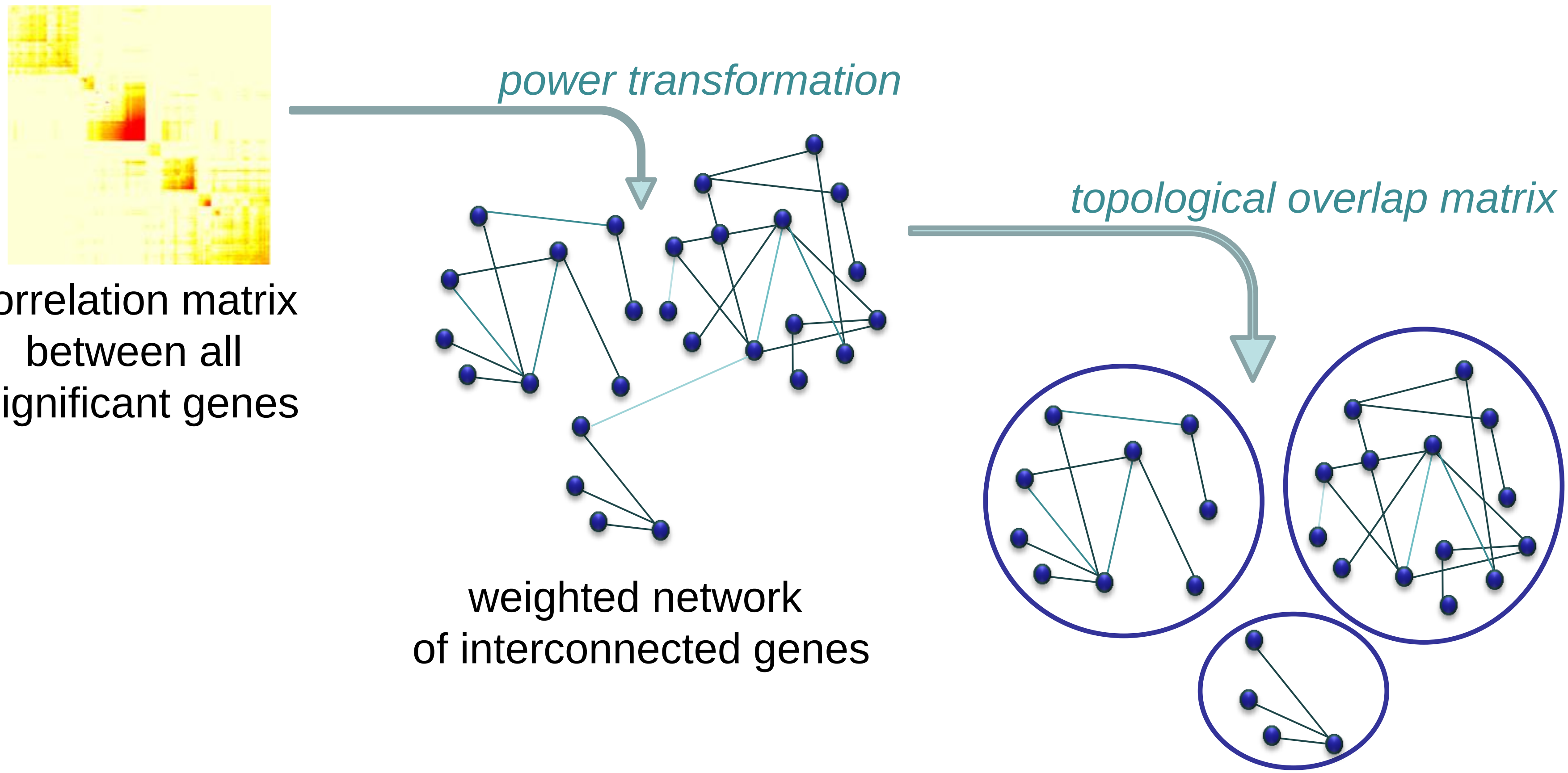
Results – *DGEA and GSEA*

- *~1,000 significant genes (FDR < 5%)*



Methods – *WGCNA*

Weighted Gene Co-expression Network Analysis (WGCNA):



correlation matrix
between all
significant genes

power transformation

weighted network
of interconnected genes

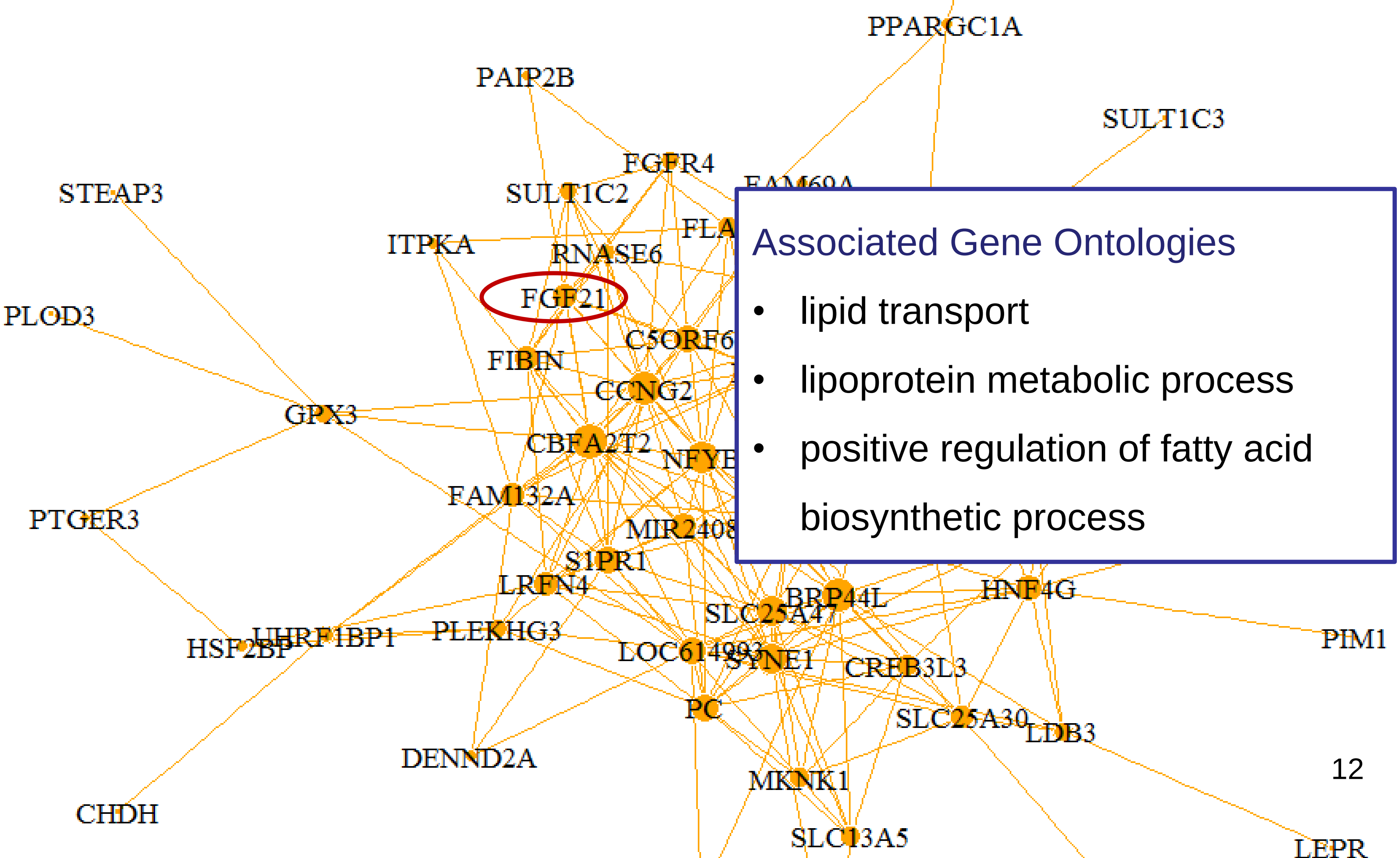
topological overlap matrix

gene modules =
set of tightly connected genes

(Langfelder and Horvarth, 2008)

Results – *WGCNA*

- *15 modules detected (~1,000 significant genes)*



Associated Gene Ontologies

- lipid transport
- lipoprotein metabolic process
- positive regulation of fatty acid biosynthetic process

Conclusions

- ~10% of the ~10,000 genes expressed in the liver are significant comparing ante- vs. post-partum

  ⇨ *importance of liver metabolism for adaptation*

- major hepatic changes involved in gluconeogenesis and lipid mobilization (*PC, FGF21, „adipocytokines signaling pathway“)*

- significant pathways *(e.g. „steroid hormone biosynthesis“)* indicate immunological changes

- identified 15 modules with different expression patterns

  ⇨ *may have different roles in the metabolic adaptation*

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swissgenetics

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References

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- ~10% of the ~10,000 genes expressed in the liver are significant comparing ante- vs. post-partum
  ⇒ *importance of liver metabolism for adaptation*
- major hepatic changes involved in gluconeogenesis and lipid mobilization (*PC, FGF21, „adipocytokines signaling pathway“)*
- significant pathways *(e.g. „steroid hormone biosynthesis“)* indicate immunological changes
- identified 15 modules with different expression patterns
  ⇒ *may have different roles in the metabolic adaptation*

RNA-Seq

Quality Control and Trimming

FastQC and Trimmomatic

Mapping on reference genome (BT, UMD3.1 rel.85)

STAR:
~ 94% Mapping-Rate
~ 22M Fragmente

Counting reads overlapping exons

featureCounts:
~ 17M Counts

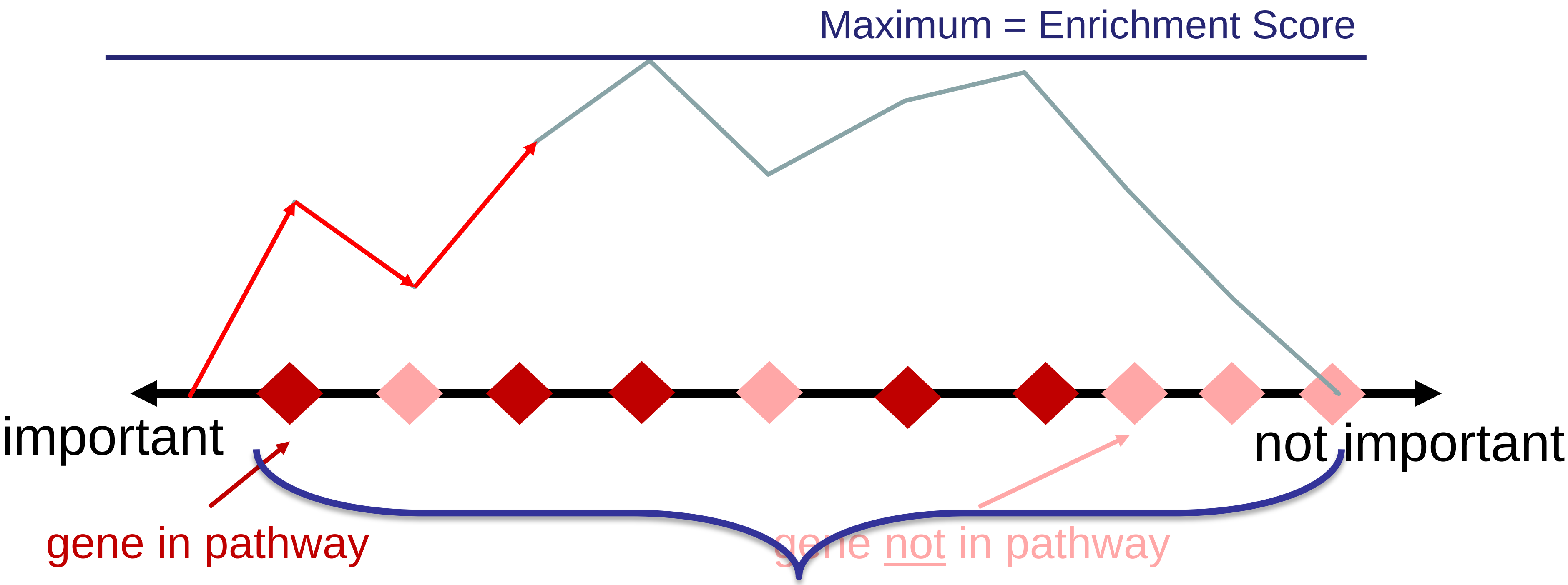
Normalization and modelling of count data

R-Paket edgeR:
TMM, GLM

DGEA, GSEA and WGCNA

GSEA

Gene-Set Enrichment Analysis (Subramanian et al., 2005)



Maximum = Enrichment Score

important

not important

gene in pathway

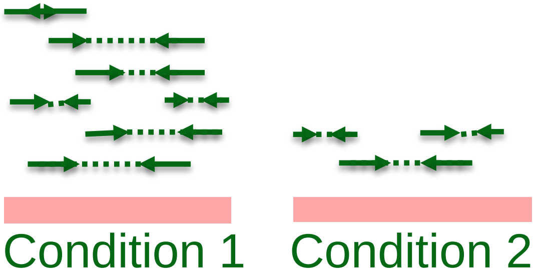
gene not in pathway

$-\log_{10}(p-value)$

Analysis of RNA-Seq Data

Results:

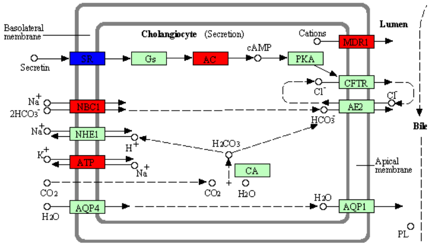
Differential Gene Expression Analysis (DGEA)



⇨

List of differentially expressed genes

Gene Set Enrichment Analysis (GSEA)

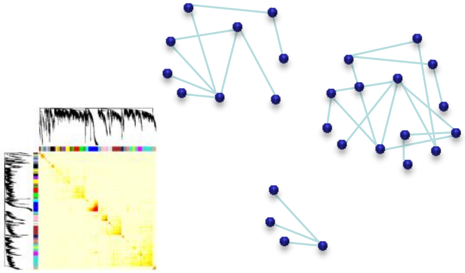


KEGG pathways

⇨

List of differentially expressed pathways

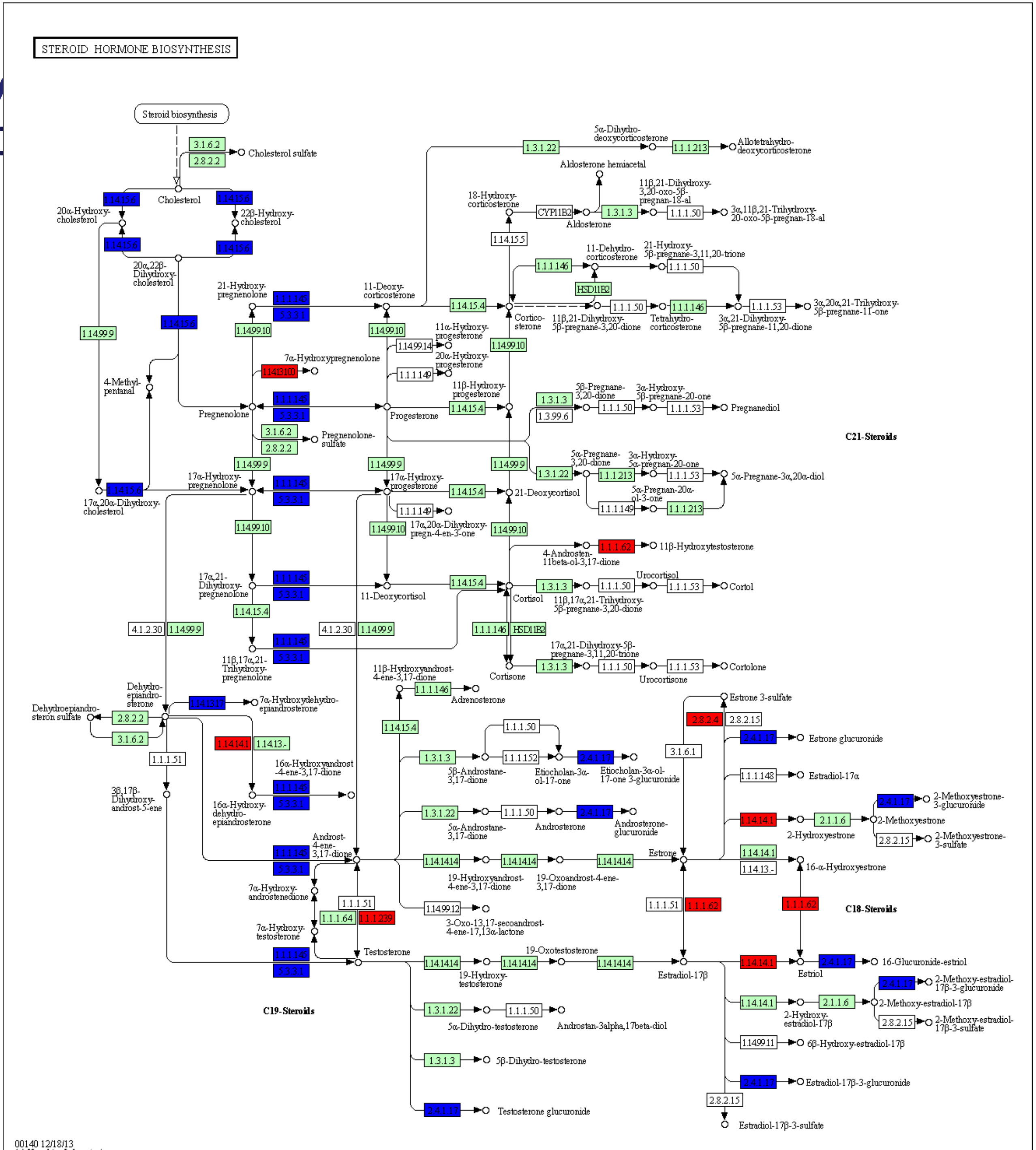
Weighted Gene Co-expression Network Analysis (WGCNA)



⇨

Sets of co-expressed genes differentially expressed

Pathw


STEROID HORMONE BIOSYNTHESIS
Steroid biosynthesis
3.1.6.2
2.8.2.2
Cholesterol sulfate
Cholesterol
1.14.15.6
20α-Hydroxy-cholesterol
22β-Hydroxy-cholesterol
20α,22β-Dihydroxy-cholesterol
1.14.99.9
4-Methyl-pentanal
21-Hydroxy-pregnenolone
1.1.1.145
5.3.3.1
1.14.99.10
7α-Hydroxypregnenolone
1.14.13.100
Pregnenolone
Pregnenolone-sulfate
17α-Hydroxy-pregnenolone
17α,20α-Dihydroxy-cholesterol
17α,21-Dihydroxy-pregnenolone
1.14.15.4
11β,17α,21-Trihydroxy-pregnenolone
4.1.2.30
11-Deoxy-corticosterone
11α-Hydroxy-progesterone
1.14.99.14
20α-Hydroxy-progesterone
1.1.1.149
Progesterone
17α-Hydroxy-progesterone
17α,20α-Dihydroxy-pregn-4-en-3-one
11-Deoxycortisol
11β-Hydroxy-progesterone
21-Deoxycortisol
5α-Dihydro-deoxycorticosterone
1.3.1.22
1.1.1.213
Allotetrahydro-deoxycorticosterone
Aldosterone hemiacetal
18-Hydroxy-corticosterone
CYP11B2
1.3.1.3
Aldosterone
11β,21-Dihydroxy-3,20-oxo-5β-pregnan-18-al
1.1.1.50
3α,11β,21-Trihydroxy-20-oxo-5β-pregnan-18-al
1.14.15.5
11-Dehydro-corticosterone
1.1.1.146
21-Hydroxy-5β-pregnane-3,11,20-trione
HSD11B2
Cortico-sterone
11β,21-Dihydroxy-5β-pregnane-3,20-dione
Tetrahydro-corticosterone
3α,21-Dihydroxy-5β-pregnane-11,20-dione
1.1.1.53
3α,20α,21-Trihydroxy-5β-pregnane-11-one
5β-Pregnane-3,20-dione
1.3.99.6
3α-Hydroxy-5β-pregnane-20-one
Pregnanediol
C21-Steroids
5α-Pregnane-3,20-dione
3α-Hydroxy-5α-pregnan-20-one
5α-Pregnane-3α,20α-diol
5α-Pregnan-20α-ol-3-one
4-Androsten-11beta-ol-3,17-dione
1.1.1.62
11β-Hydroxytestosterone
Cortisol
Urocortisol
Cortol
11β,17α,21-Trihydroxy-5β-pregnane-3,20-dione
17α,21-Dihydroxy-5β-pregnane-3,11,20-trione
Cortisone
Urocortisone
Cortolone
11β-Hydroxyandrost-4-ene-3,17-dione
Adrenosterone
Dehydro-epiandro-sterone
Dehydroepiandro-steron sulfate
1.14.13.17
7α-Hydroxydehydro-epiandrosterone
1.14.14.1
1.14.13.-
1.1.1.51
3β,17β-Dihydroxy-androst-5-ene
16α-Hydroxyandrost-4-ene-3,17-dione
16α-Hydroxy-dehydro-epiandrosterone
Androst-4-ene-3,17-dione
7α-Hydroxy-androstenedione
7α-Hydroxy-testosterone
1.1.1.64
1.1.1.239
Testosterone
C19-Steroids
5β-Androstane-3,17-dione
1.1.1.152
Etiocholan-3α-ol-17-one
2.4.1.17
Etiocholan-3α-ol-17-one 3-glucuronide
5α-Androstane-3,17-dione
Androsterone
Androsterone-glucuronide
1.14.14.14
19-Hydroxyandrost-4-ene-3,17-dione
19-Oxoandrost-4-ene-3,17-dione
1.14.99.12
3-Oxo-13,17-secoandrost-4-ene-17,13α-lactone
19-Hydroxy-testosterone
19-Oxotestosterone
Estradiol-17β
5α-Dihydro-testosterone
Androstan-3alpha,17beta-diol
5β-Dihydro-testosterone
Testosterone glucuronide
Estrone 3-sulfate
2.8.2.4
2.8.2.15
3.1.6.1
Estrone glucuronide
1.1.1.148
Estradiol-17α
2.1.1.6
2-Hydroxyestrone
2-Methoxyestrone
2-Methoxyestrone-3-glucuronide
2-Methoxyestrone-3-sulfate
Estrone
16-α-Hydroxyestrone
C18-Steroids
Estriol
16-Glucuronide-estriol
2-Hydroxy-estradiol-17β
2-Methoxy-estradiol-17β
2-Methoxy-estradiol-17β-3-glucuronide
2-Methoxy-estradiol-17β-3-sulfate
1.14.99.11
6β-Hydroxy-estradiol-17β
Estradiol-17β-3-glucuronide
Estradiol-17β-3-sulfate
00140 12/18/13

Mappen der Reads – STAR vs Tophat2

Reference genome und gene annotation:

- *Ensembl UMD3.1 Release 85*
- *24‘616 genes (19‘994 coding)*

STAR	**Tophat2**
• fast (~20 min)	• slow (~12 h)
• suggested by *GATK Best Practices*	• high mapping rate (Kim et. al., 2013)
• high mapping rate (Engström et al., 2013)	• high rate of false positives (Dobin and Gingeras, 2013)
• *Mapping-Rate: ~94%*	• *Mapping-Rate: ~81%*

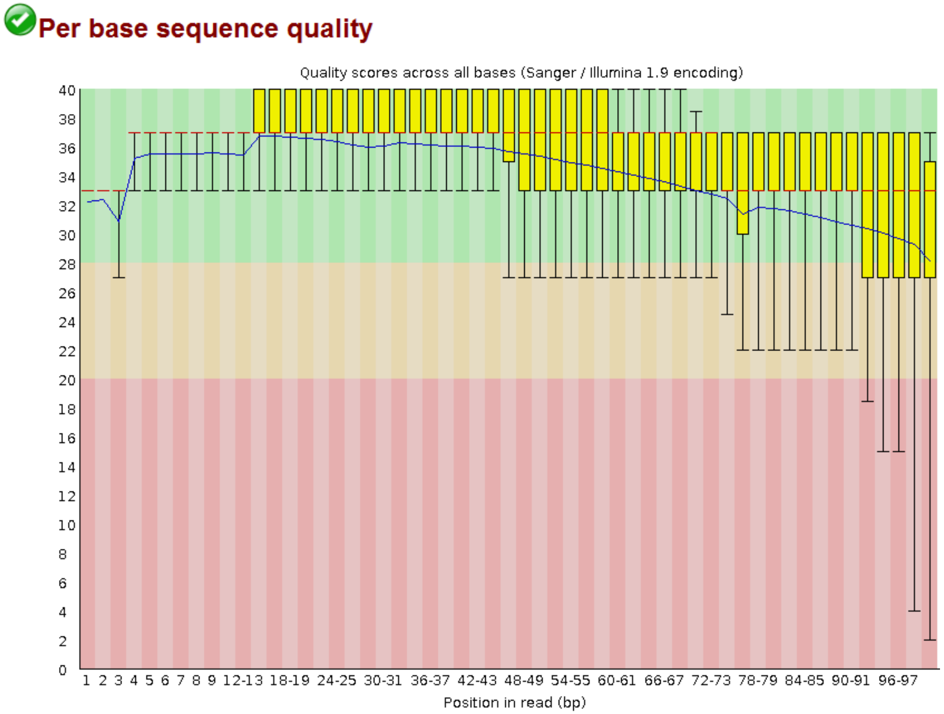
In our study minor differences

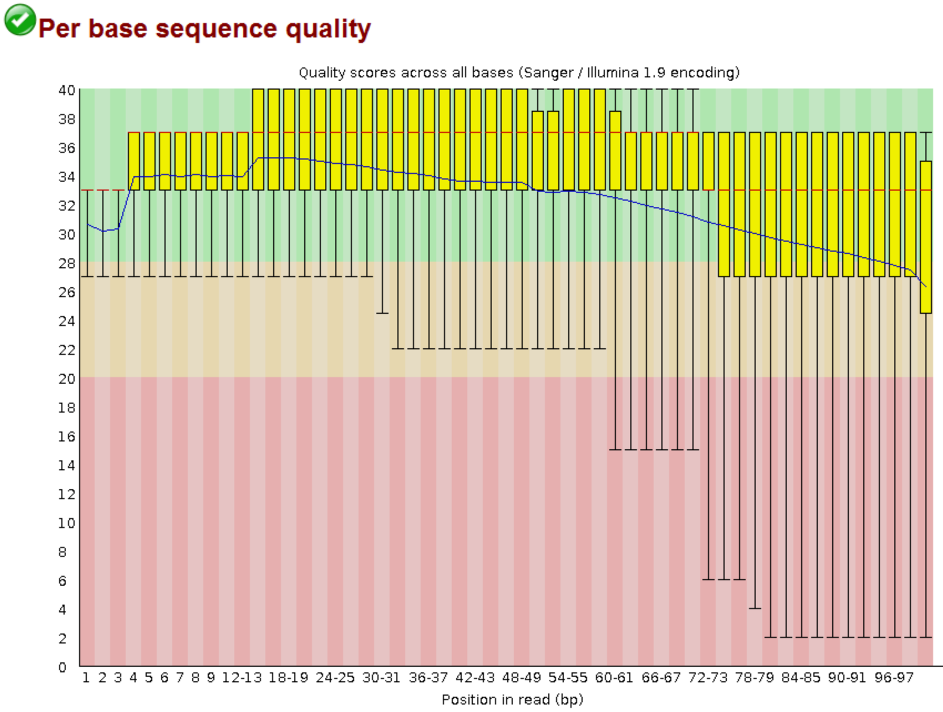
Qualitätskontrolle – FastQC

RNA fragment

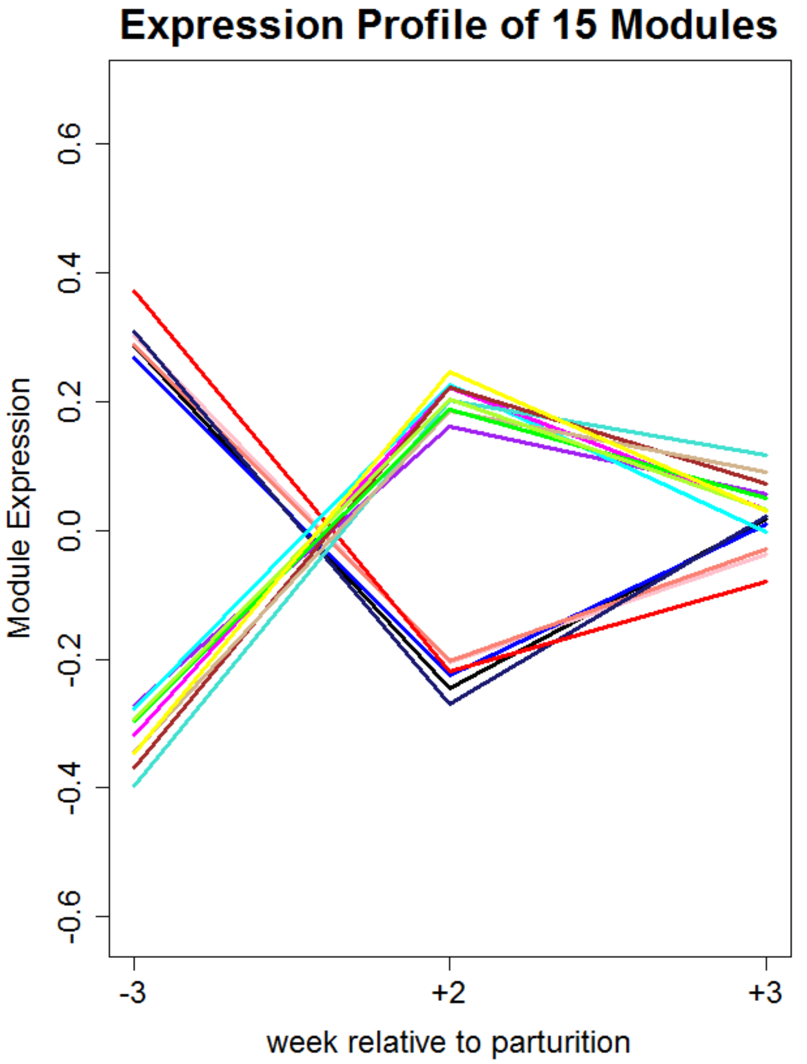
Forward Read: 101 bp

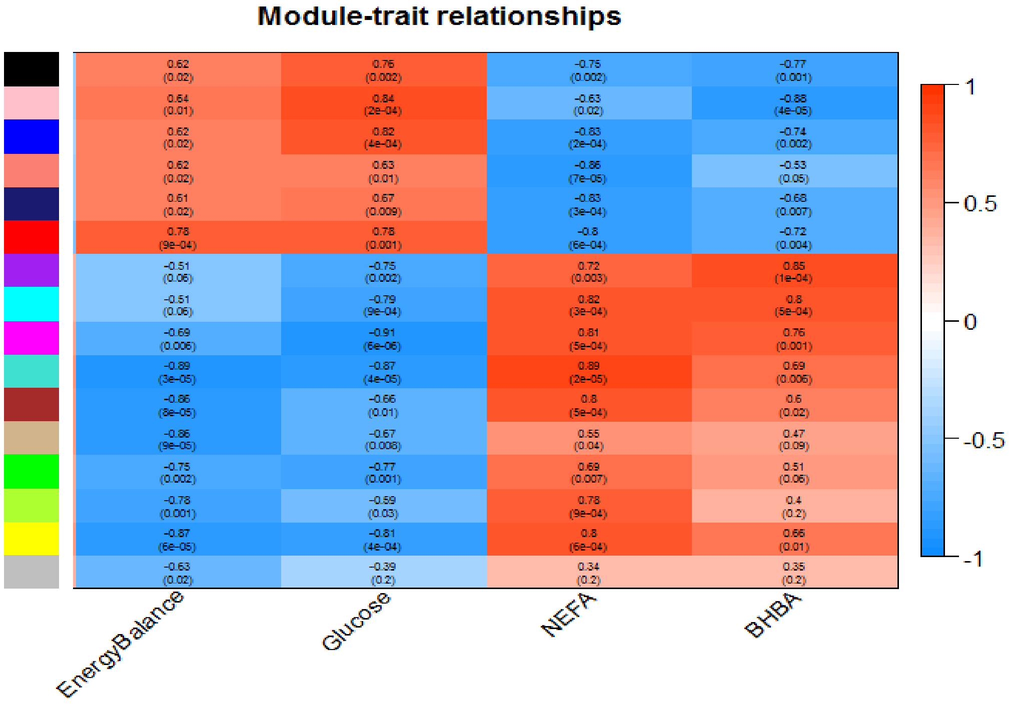
Reverse Read: 101 bp





Results





	EnergyBalance	Glucose	NEFA	BHBA
⬛	0.62 (0.02)	0.76 (0.002)	−0.75 (0.002)	−0.77 (0.001)
🟥	0.64 (0.01)	0.84 (2e−04)	−0.63 (0.02)	−0.88 (4e−05)
🟦	0.62 (0.02)	0.82 (4e−04)	−0.83 (2e−04)	−0.74 (0.002)
🟥	0.62 (0.02)	0.63 (0.01)	−0.86 (7e−05)	−0.53 (0.05)
🟦	0.61 (0.02)	0.67 (0.009)	−0.83 (3e−04)	−0.68 (0.007)
🟥	0.78 (9e−04)	0.78 (0.001)	−0.8 (6e−04)	−0.72 (0.004)
🟪	−0.51 (0.06)	−0.75 (0.002)	0.72 (0.003)	0.85 (1e−04)
🟦	−0.51 (0.06)	−0.79 (9e−04)	0.82 (3e−04)	0.8 (5e−04)
🟪	−0.69 (0.006)	−0.91 (6e−06)	0.81 (5e−04)	0.76 (0.001)
🟩	−0.89 (3e−05)	−0.87 (4e−05)	0.89 (2e−05)	0.69 (0.006)
🟫	−0.86 (8e−05)	−0.66 (0.01)	0.8 (5e−04)	0.6 (0.02)
🟫	−0.86 (9e−05)	−0.67 (0.008)	0.55 (0.04)	0.47 (0.09)
🟩	−0.75 (0.002)	−0.77 (0.001)	0.69 (0.007)	0.51 (0.06)
🟩	−0.78 (0.001)	−0.59 (0.03)	0.78 (9e−04)	0.4 (0.2)
🟨	−0.87 (6e−05)	−0.81 (4e−04)	0.8 (6e−04)	0.66 (0.01)
⬜	−0.63 (0.02)	−0.39 (0.2)	0.34 (0.2)	0.35 (0.2)