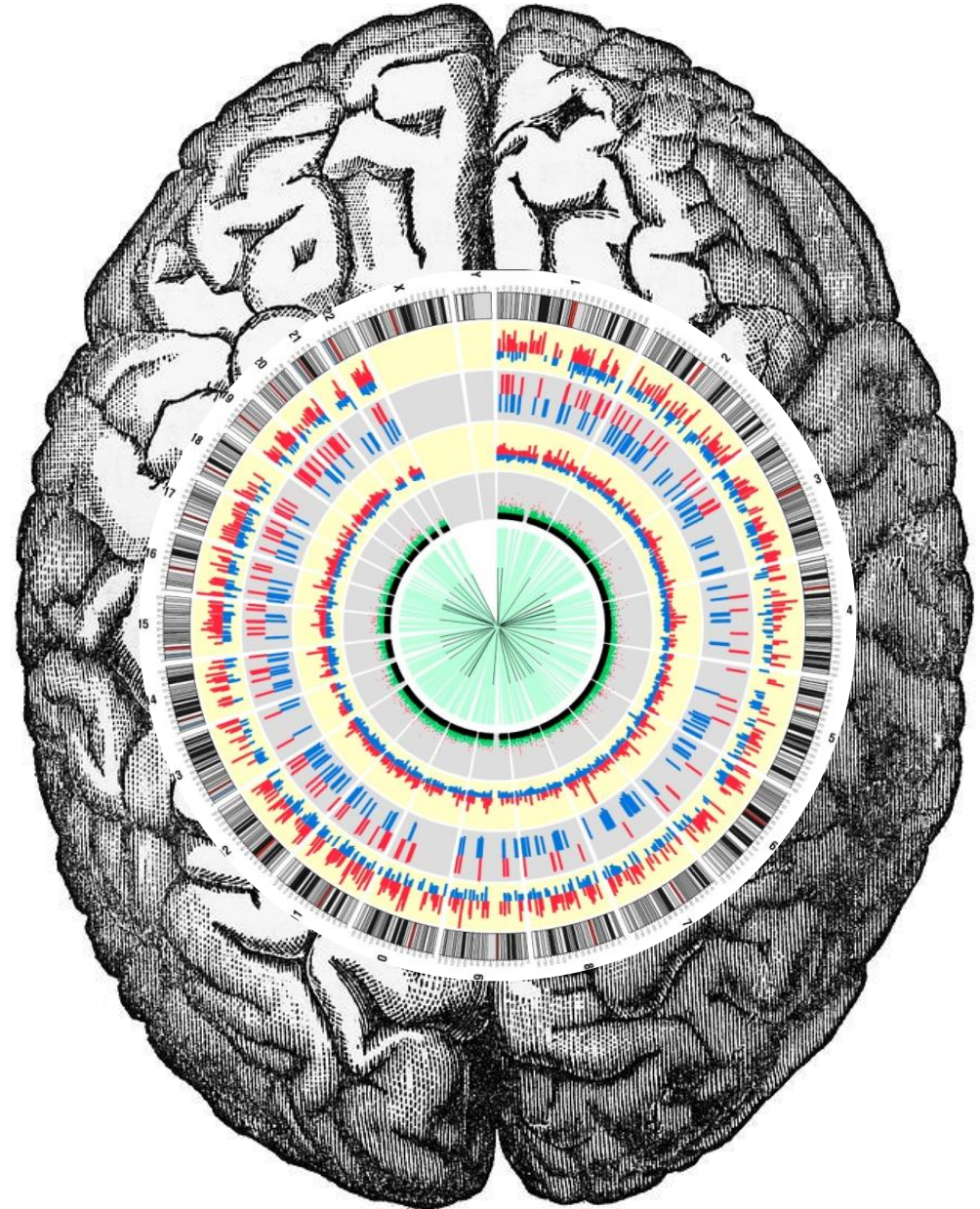


# Systems Genomics FS22 – Group 6

Lukas Radtke  
Ilya Schneider  
Moritz Ullhofen

21. Dec 2022, Basel



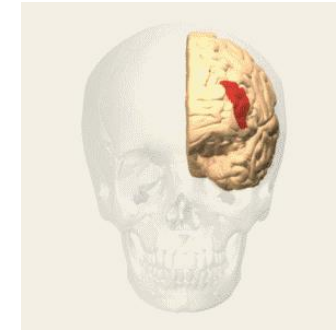
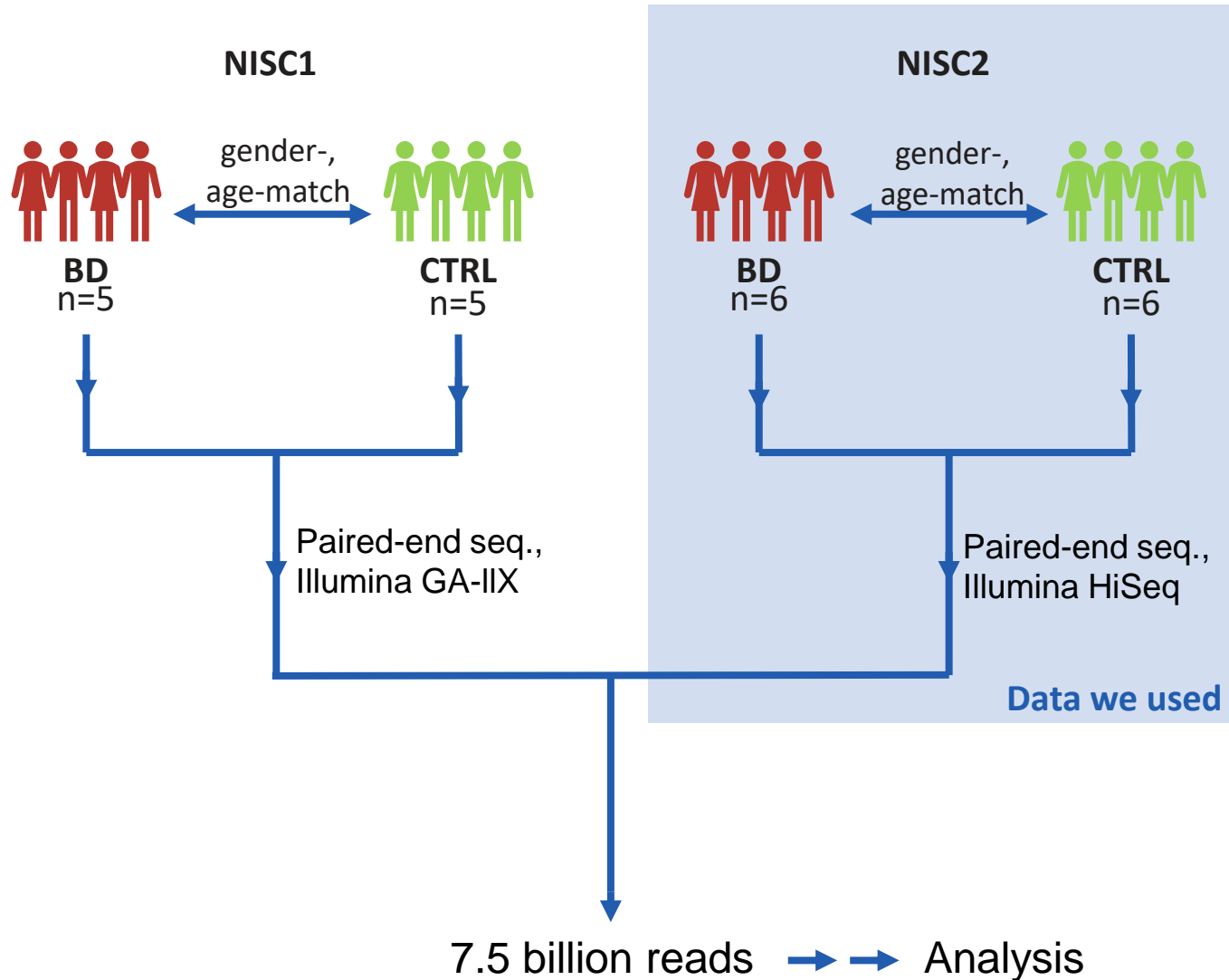
# The Paper – Akula et al. 2014

## RNA-sequencing of the brain transcriptome implicates dysregulation of neuroplasticity, circadian rhythms and GTPase binding in bipolar disorder.

N Akula, J Barb, X Jiang, JR Wendland, KH Choi, SK Sen, L Hou, DTW Chen, G Laje, K Johnson, BK Lipska, JE Kleinman, H Corrada-Bravo, S Detera-Wadleigh, PJ Munson and FJ McMahon



# The Paper – Sample



Dorsolateral Prefrontal Cortex (DLPFC)

- Bipolar Disease vs. Control
- Groups are age- & gender matched
- Two cohorts (NISC1 & NISC2)
- Tissue-samples were taken post-mortem
- Bulk-RNAseq
- Gene = all exons belonging to any isoform
- Transcript = all exons belonging to one isoform

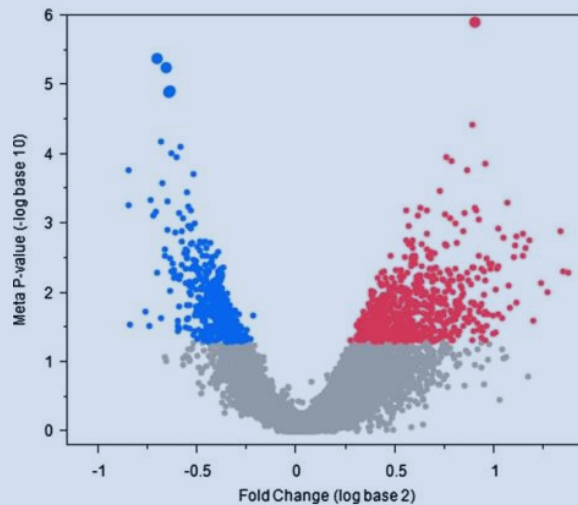
# The Paper – Results

## Read Depth, Mapping, Basic Stats

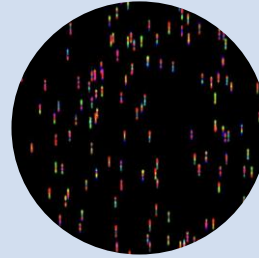
- 87% of reads mapped to the human genome build 19 Ensemble transcriptome
- PC1 & PC2 accounted for 67% variability

## Meta Analysis:

- 1225 DEGs ( $p < 0.05$ )
- 5 DE genes ( $q < 0.05$ ):
  - [LINC00173](#) (lncRNA)
  - [Prominin 1](#)
  - [ABCG2](#)
  - [A2M](#)
  - [FLI1](#)



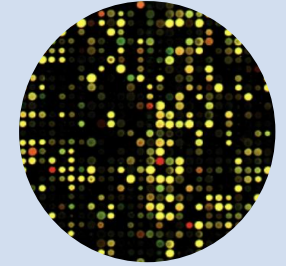
## Confirmation Methods:



Nanostring nCounter  
(41 genes)



qPCR  
(15 DEGs, 13 concordant)



Microarray  
(7579 genes,  $r = 0.42$  for DEGs)

## Gene Ontology:

- transmembrane receptor protein phosphatase activity ( $> 5$  fold)
- enrichment in 'regulation of transmission of nerve impulse ( $> 2$  fold)
- GTPase binding
- regulation of cyclic nucleotide metabolic process
- cell part morphogenesis

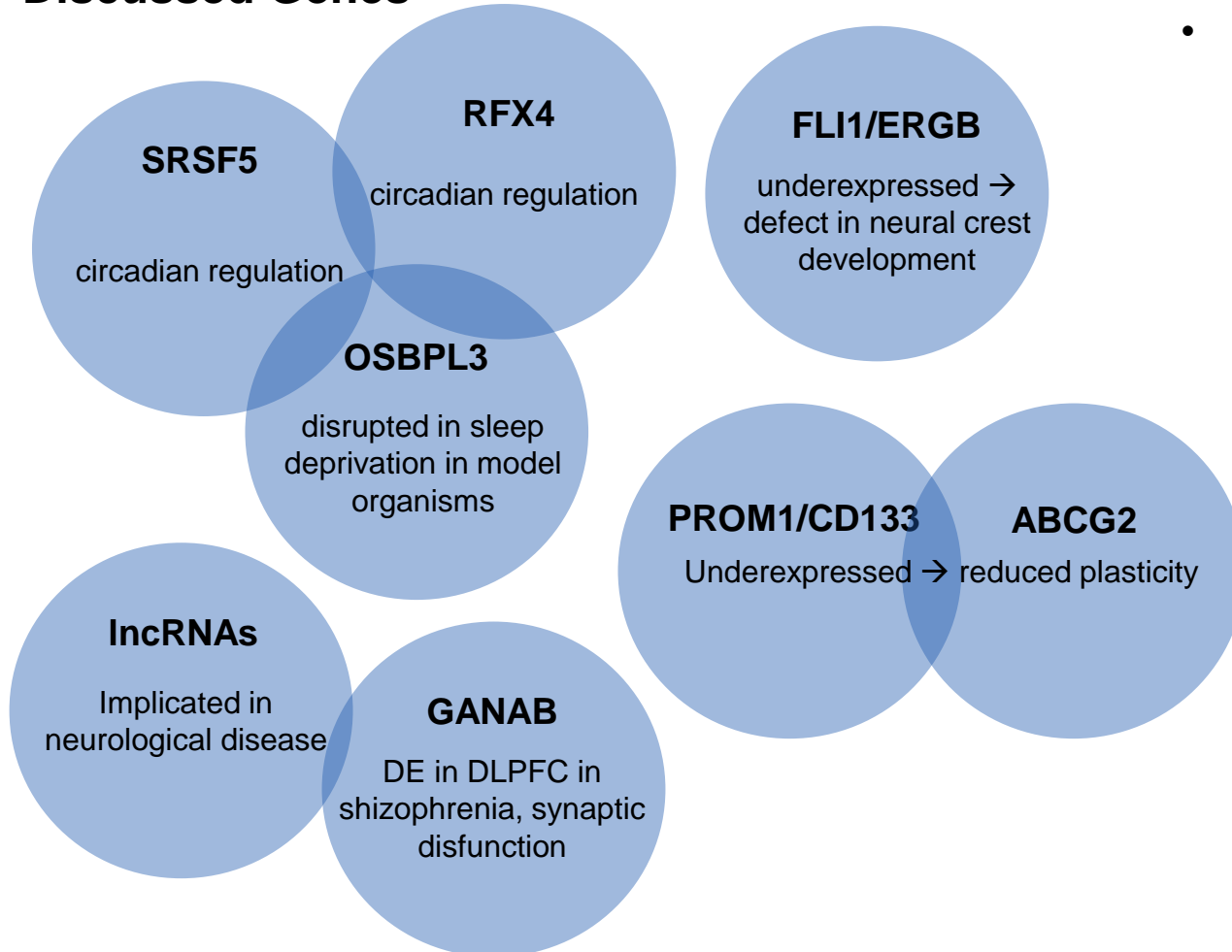
## GWAS:

- To distinguish cause and consequence
- Found enrichment of SNPs in genes of the 5 ontological categories



# The Paper – Discussion and Limitations

## Discussed Genes



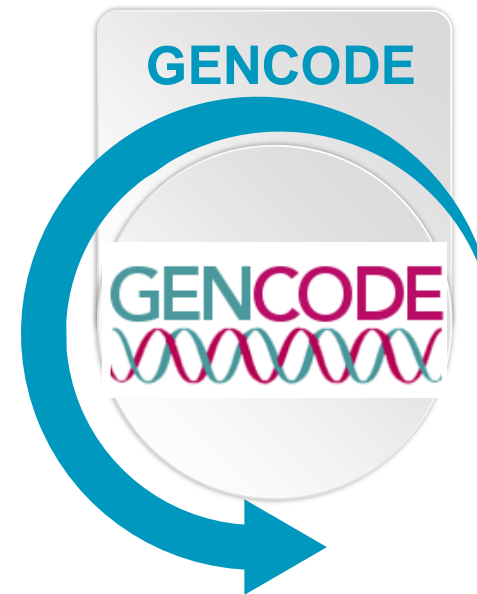
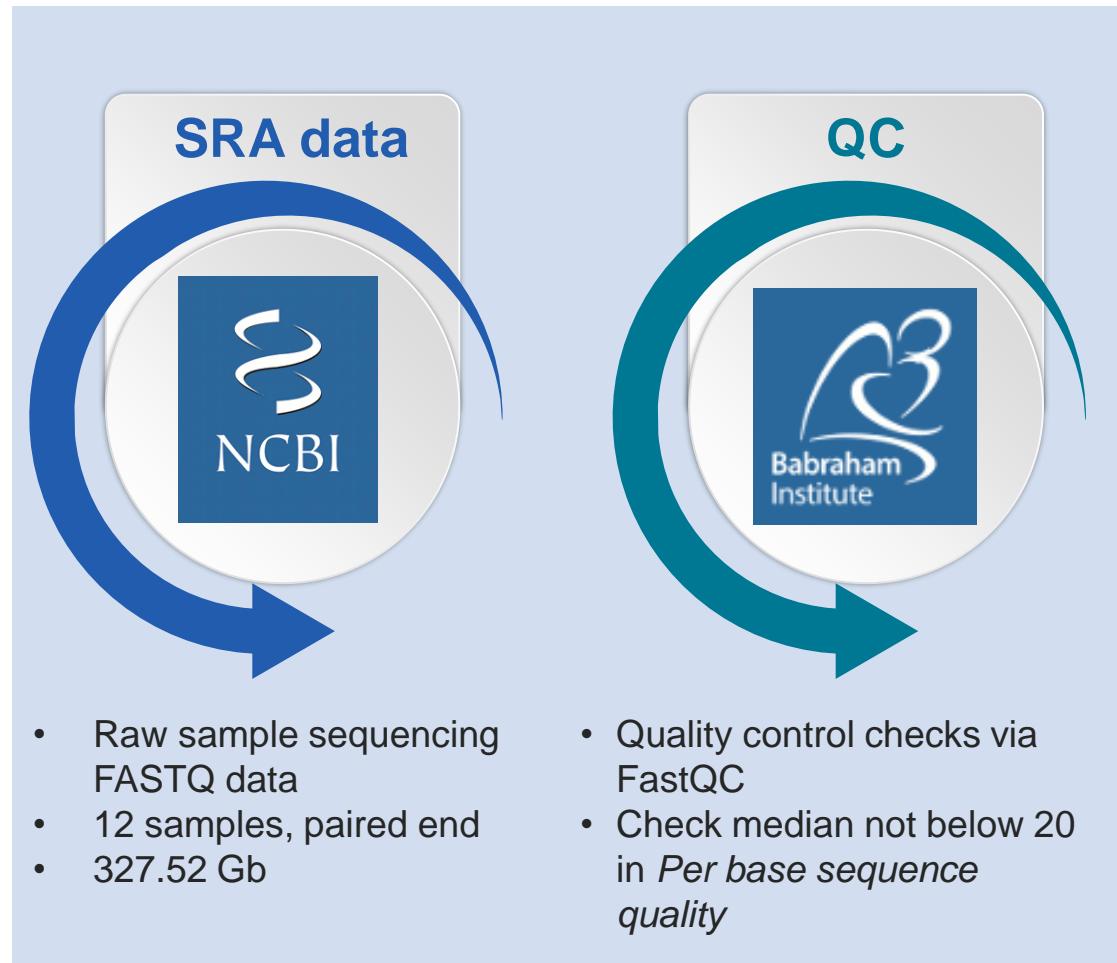
## Novelty

- Constituted the first RNAseq profiling of BD brain-transcriptome

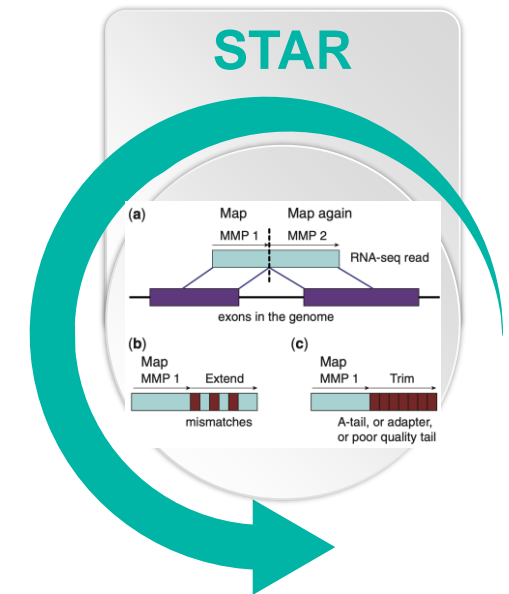
## Limitations

- No exon level analysis was conducted (differential splicing)
- Subject to agonal and post-mortem variables
- Individual differences
- Medication treatment
- No attempt to differentiate RNA of different cell types in DLPFC

# Preprocessing Workflow

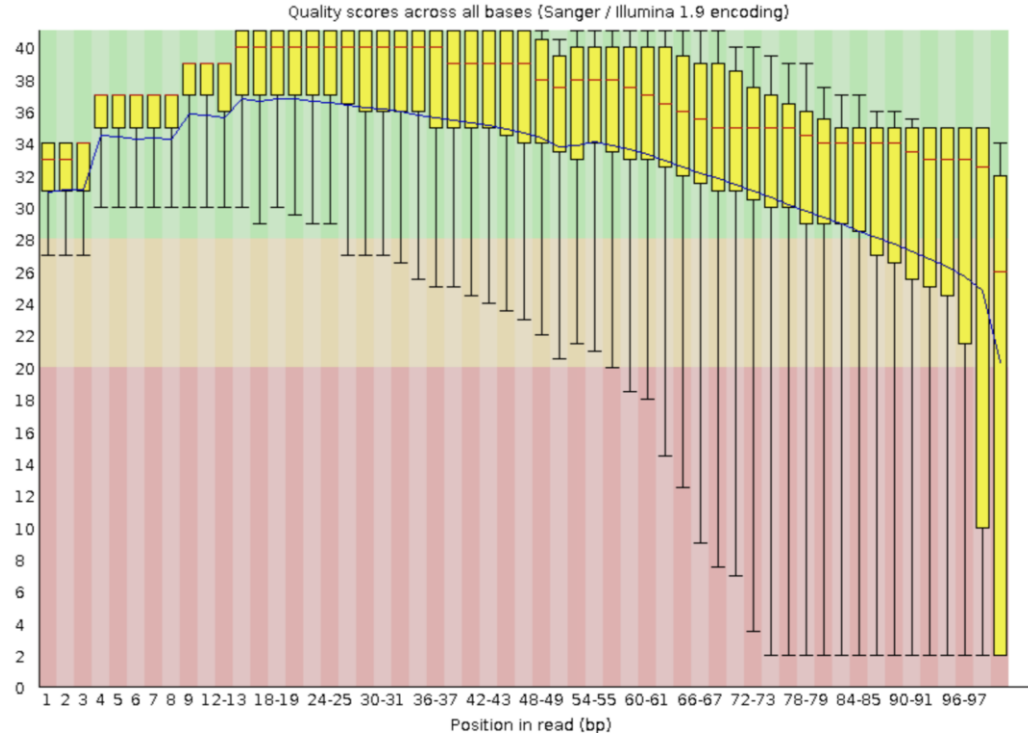


- Download newest version human genome
- Release 42, Genome assembly version: GRCh38.p13

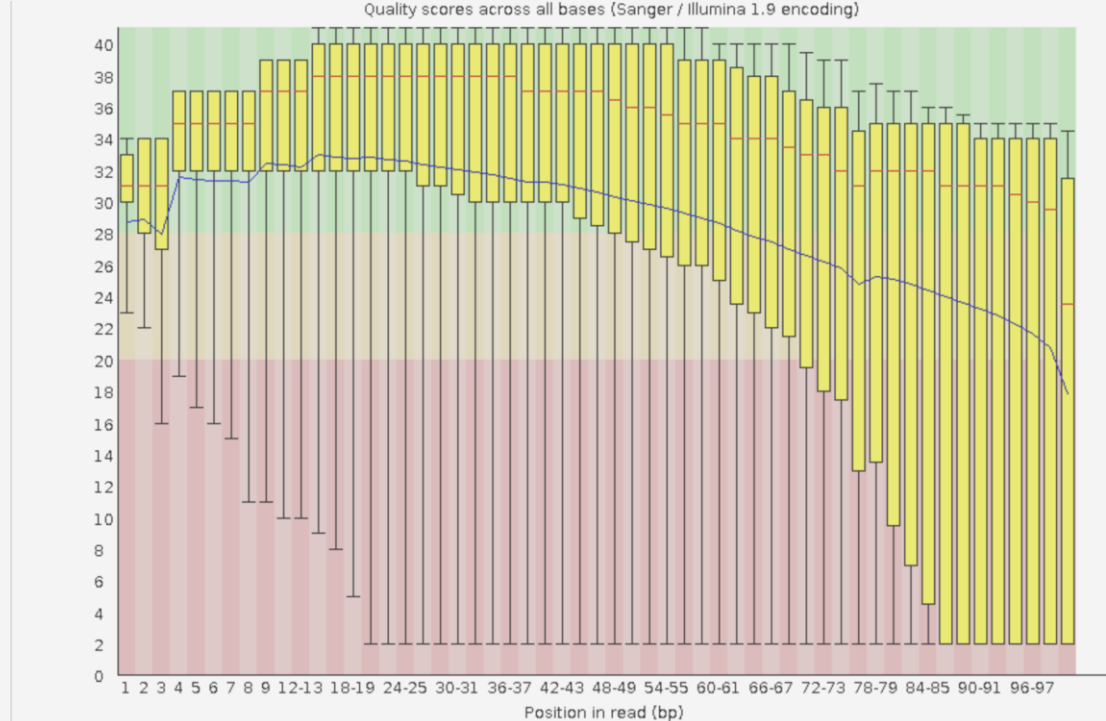
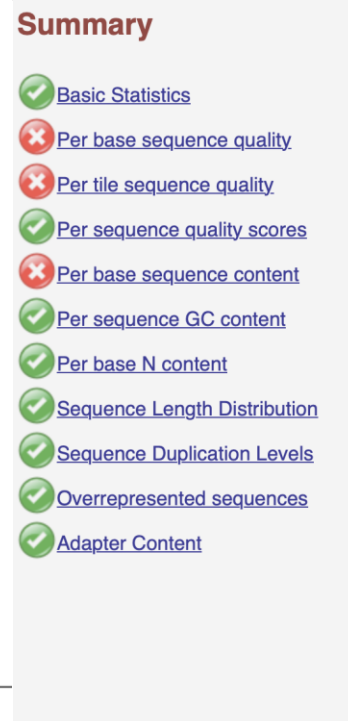


- Read Mapping
- RSEM gene expression quantification

# Quality Control



Average QC result (SRR1047871\_2)



Worst QC result (SRR1047863\_1)

- Per base sequence quality median locus never below 30, except last 1-2 bases
- Worst QC: last 15 bases low quality, but median above QC 20
- No trimming or deduplication: STAR soft-trimming

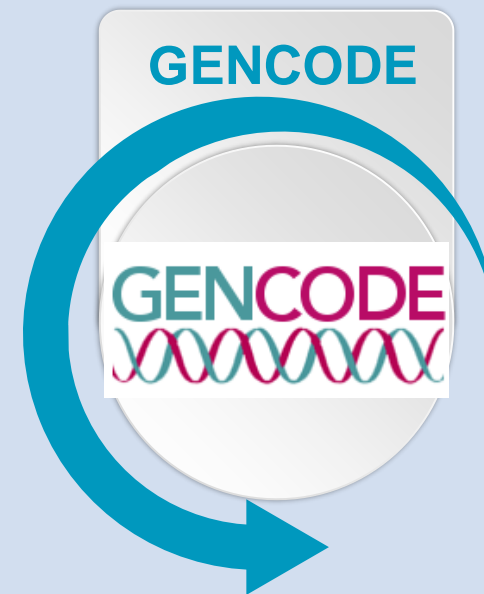
# Preprocessing Workflow



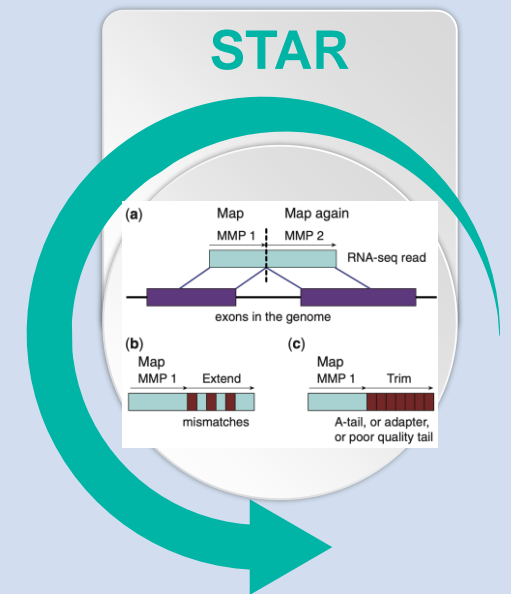
- Raw sample sequencing FASTQ data
- 12 samples, paired end
- 327.52 Gb



- Quality control checks via FastQC
- Check median not below 20 in *Per base sequence quality*



- Download newest version human genome
- Release 42, Genome assembly version: GRCh38.p13



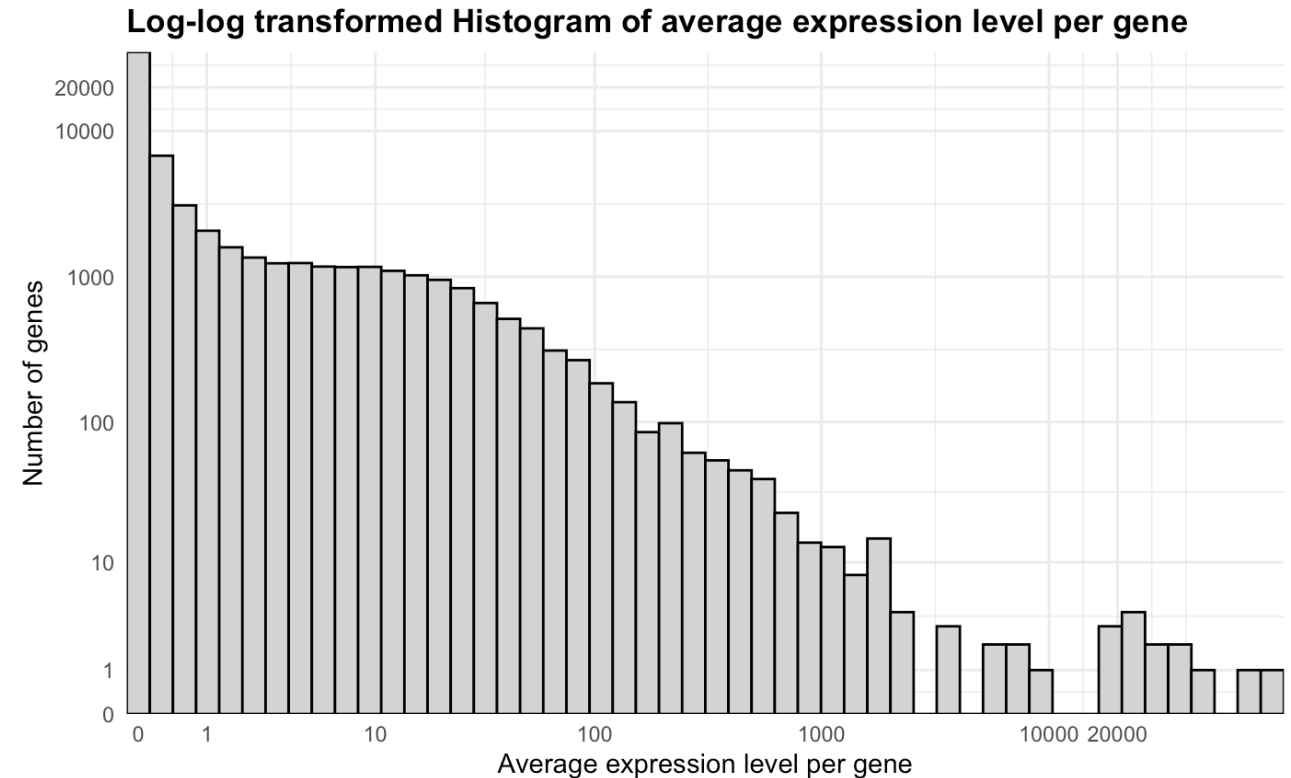
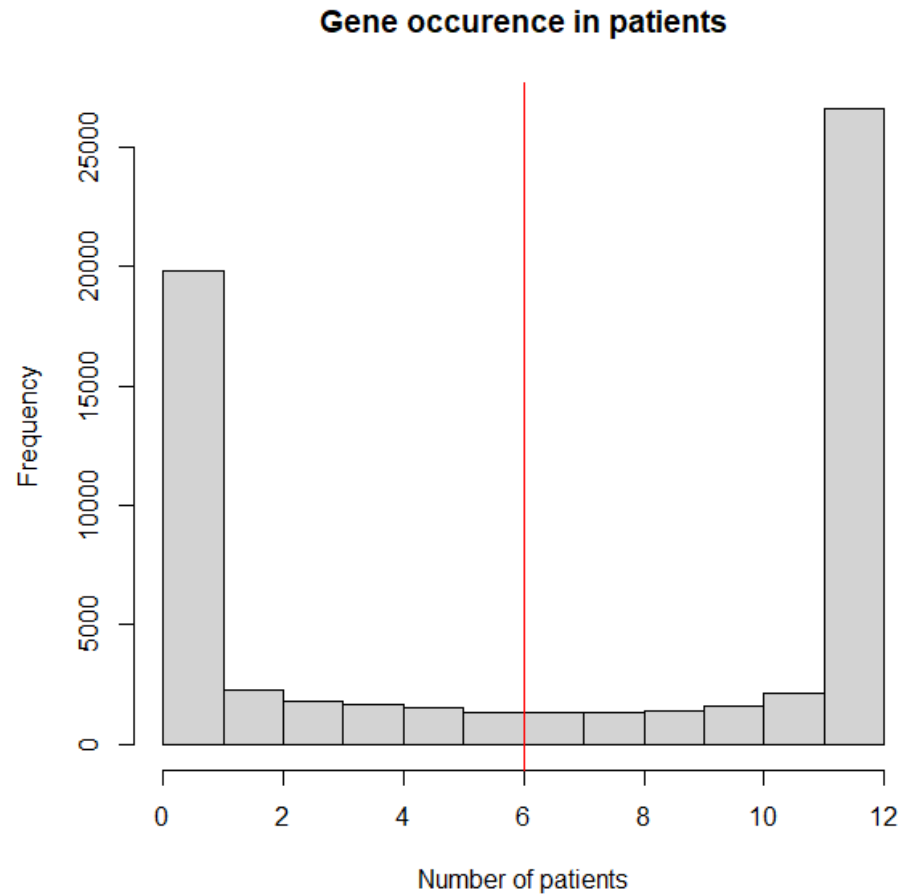
- Read Mapping
- RSEM gene expression quantification



# Preprocessing - with its challenges

- **SRA dataset download:**
  - very large dataset 327.52 Gb
  - *even with multiple cores on EULER, up to 7 hours download*
- **Reference human genome:**
  - create genome index (Release 42, Genome assembly version: GRCh38.p13)
  - *problems with creating index, newest release (19.10.2022)*
- **Read mapping and RSEM gene expression quantification:**
  - map transcriptome to human genome
    - Count number of reads aligned to each exon
    - Normalization via TPM (Transcript Per Million reads)
  - Quantification of gene expression per sample (RSEM)
    - RSEM : effective gene length = weighted average of all its isoforms
- *Script for mapping and RSEM took 44 hours with 200 GB RAM on EULER; 4 tries to run completely*

# Transcriptomic Profile Across Samples

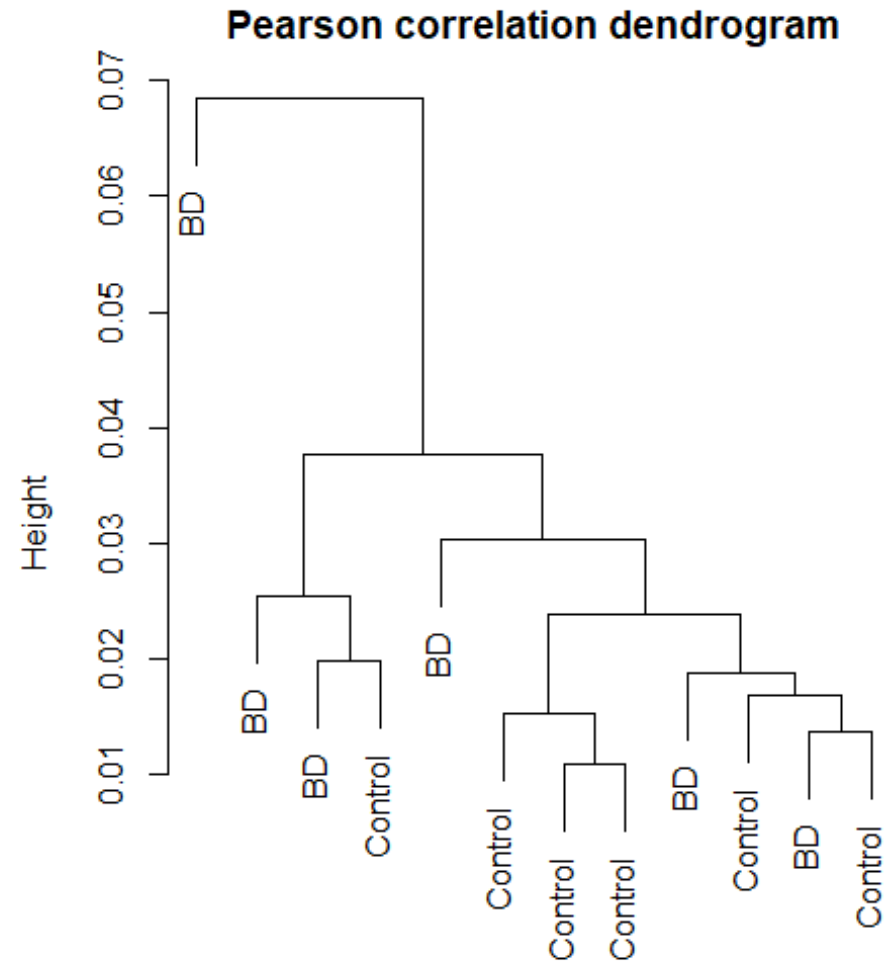
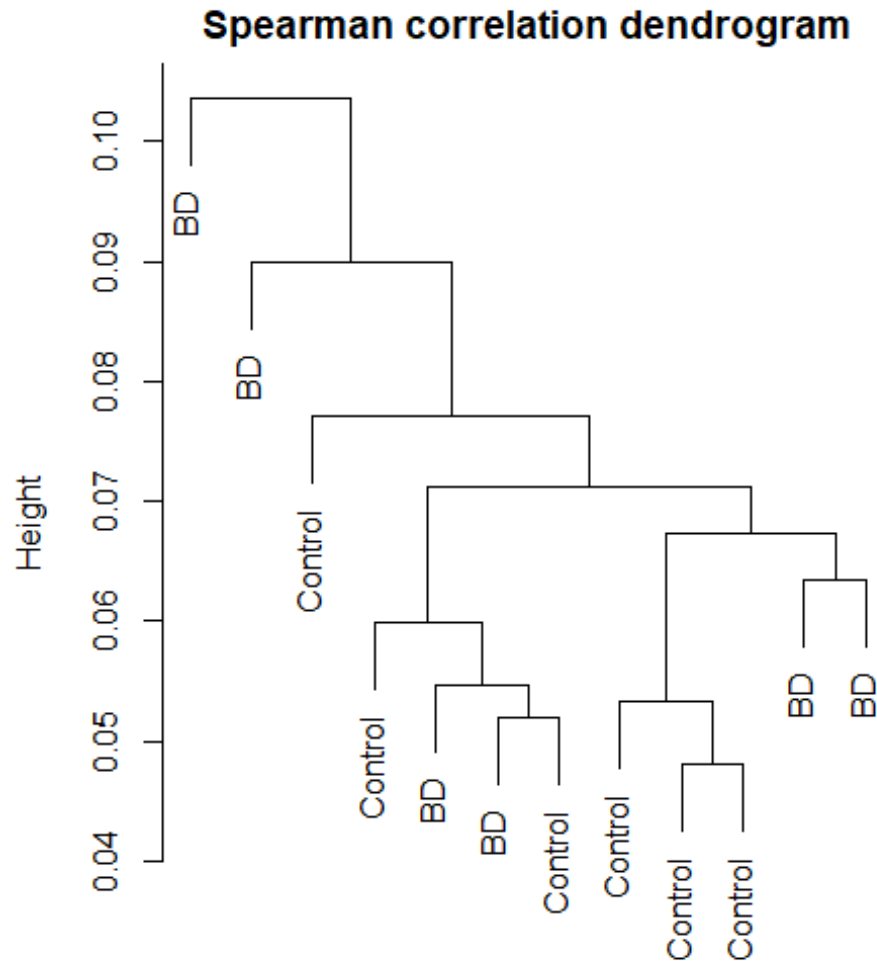


62 696 genes among 12 patients

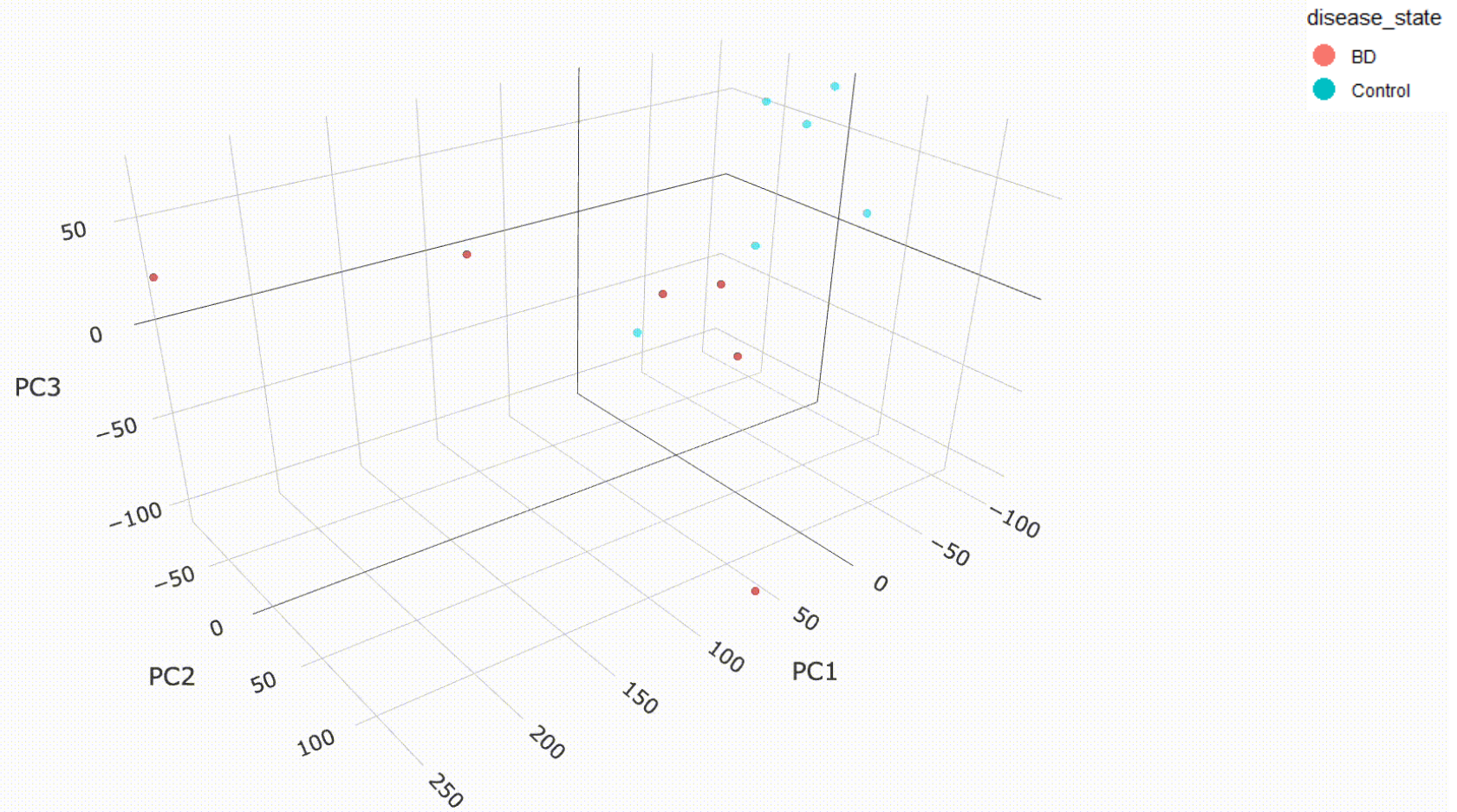
threshold: genes in at least half of samples &  $\text{TPM} \geq 1$

**35 828 genes selected for further analysis**

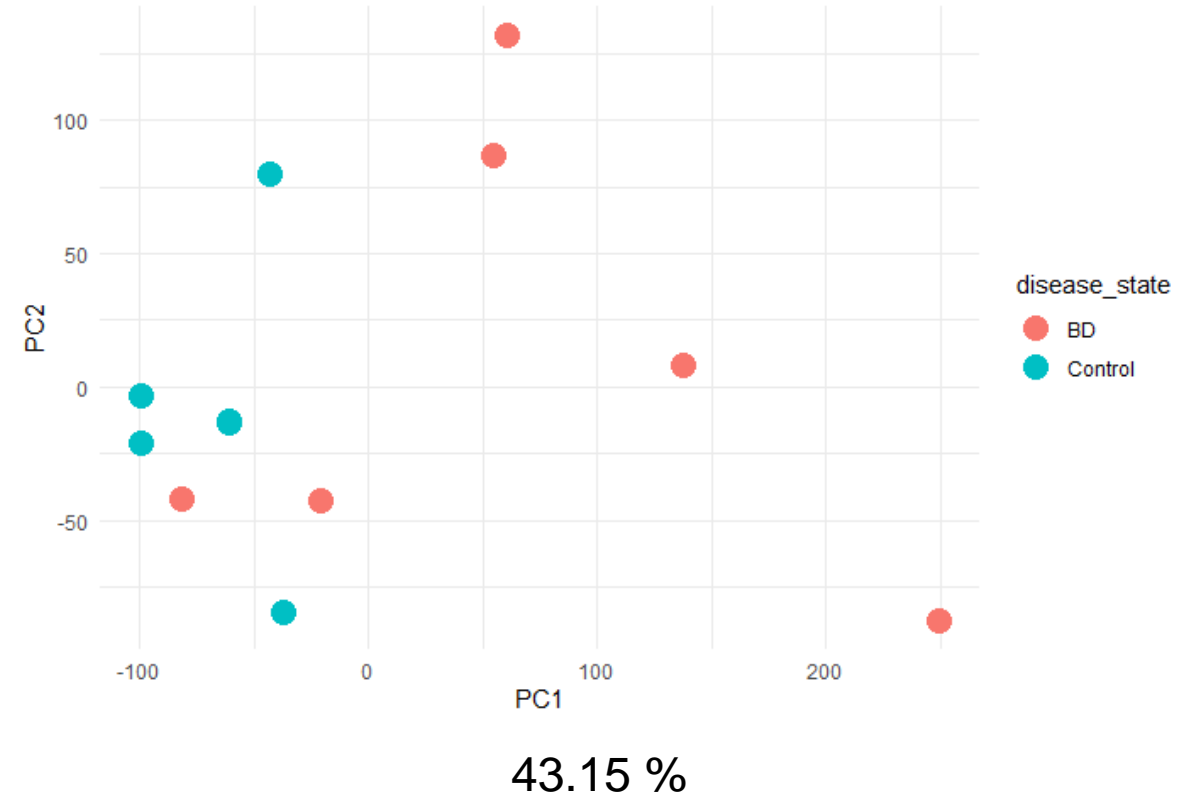
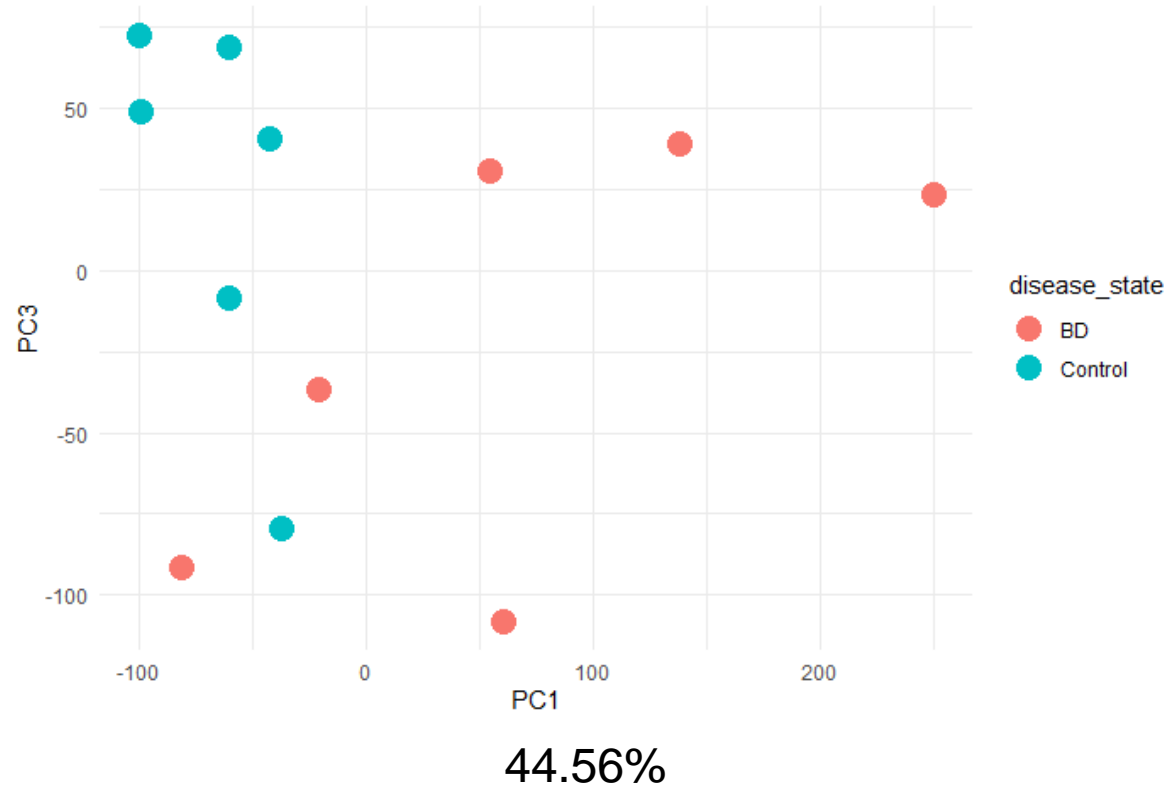
# Patient clustering in terms of gene expression



# Data Analysis - PCA



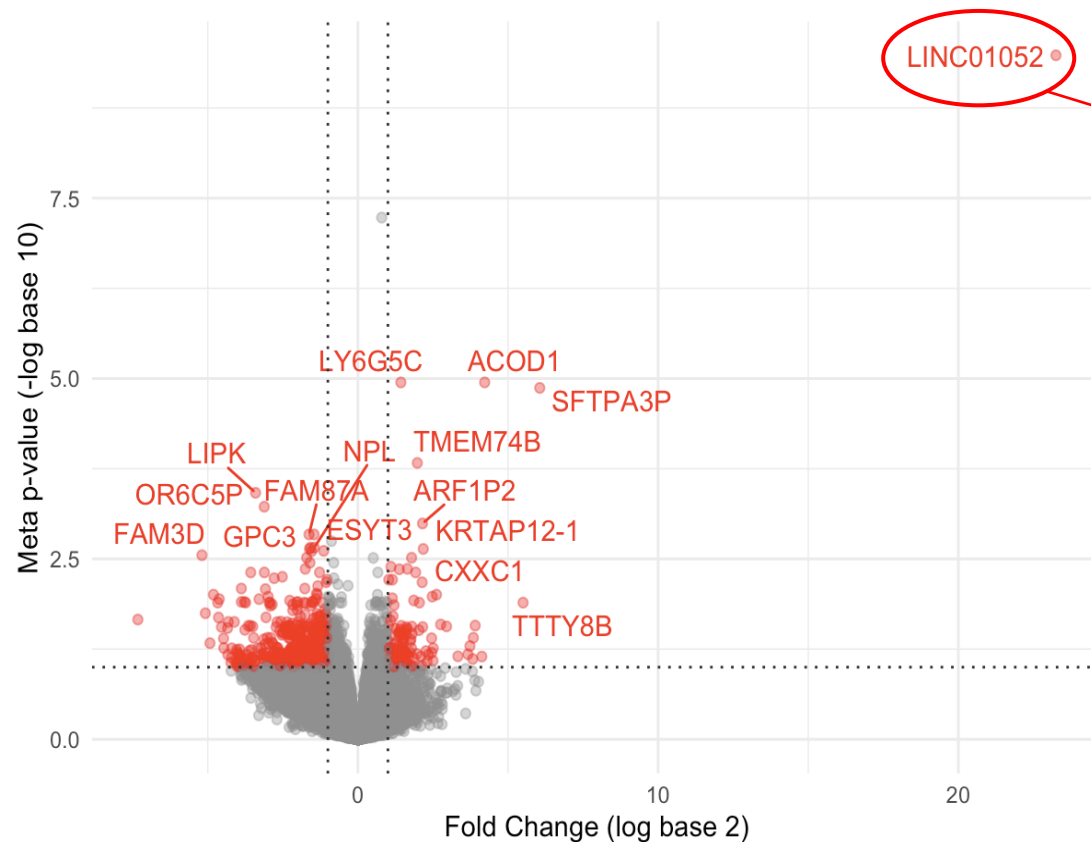
# PCA with Most Variance





# Differential Expressed Analysis

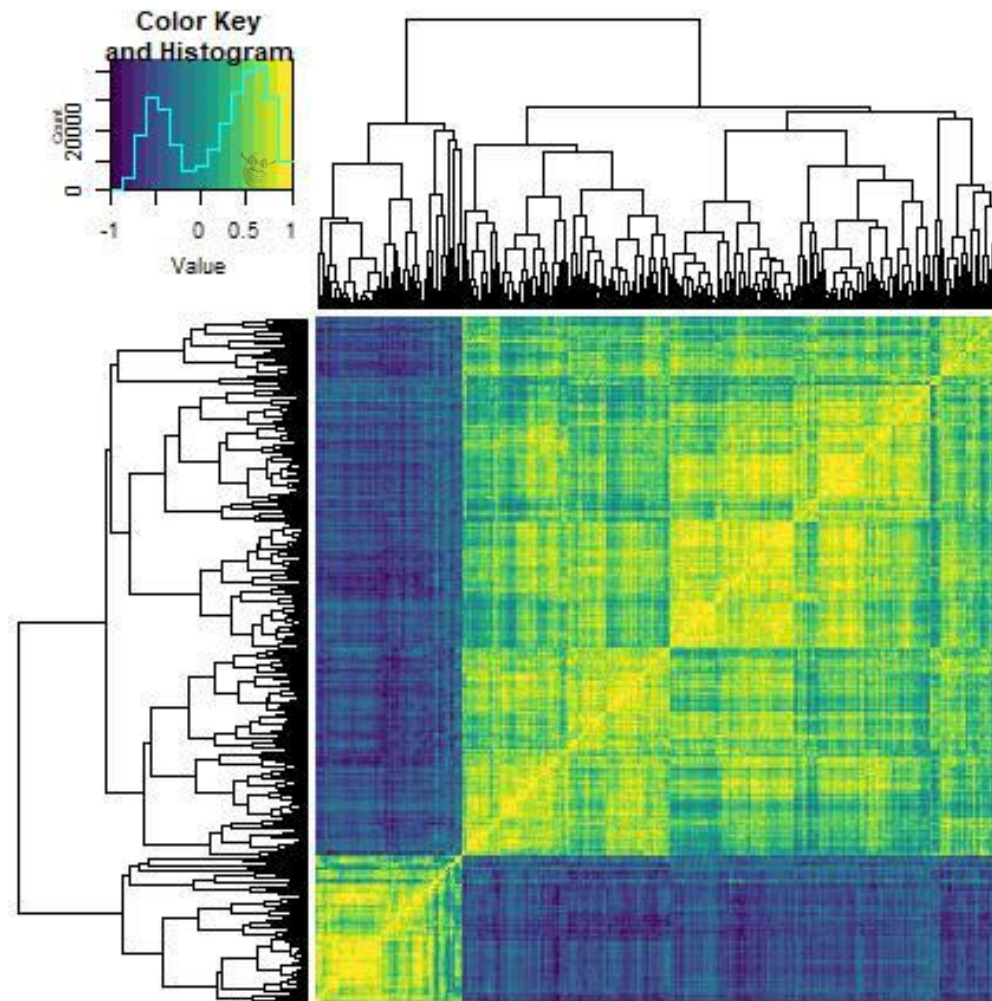
- ANOVA: 0 differentially expressed genes (DEGs)
- DeSeq2: **549 DEGs**



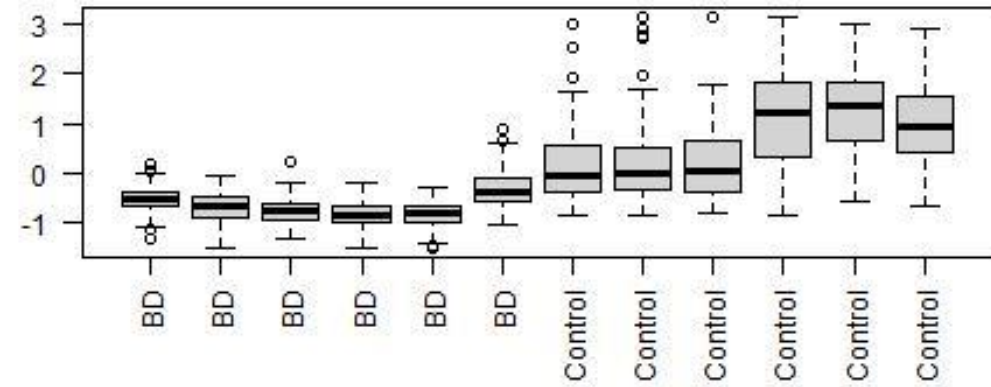
lncRNA associated with schizophrenia

Akula *et al.*, 2014  
emphasize importance of upregulated lncRNA in BD patients

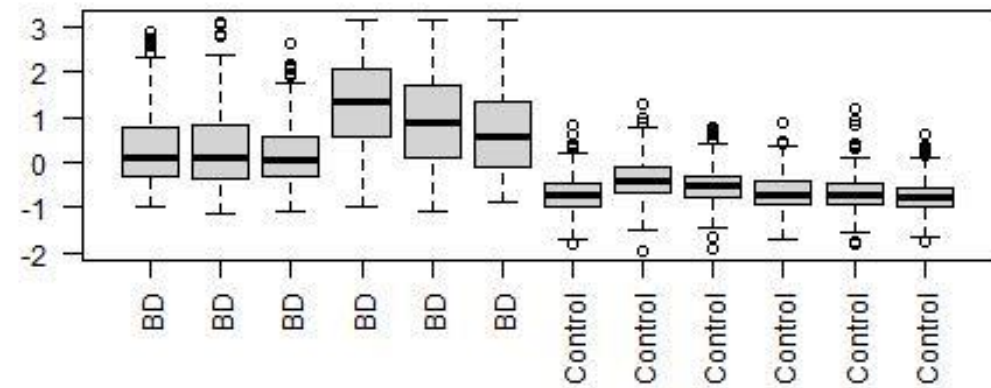
# Grouping of DEGs



**Cluster 1 (117)**

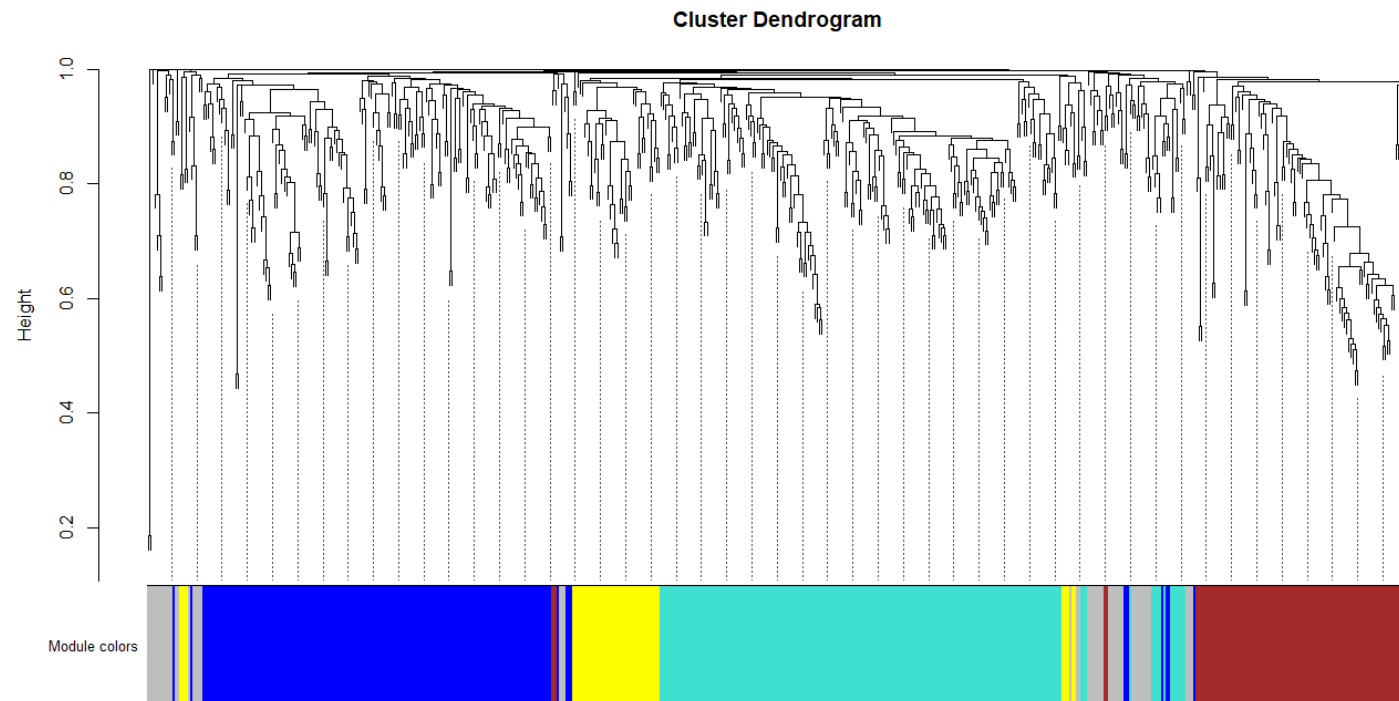


**Cluster 2 (432)**



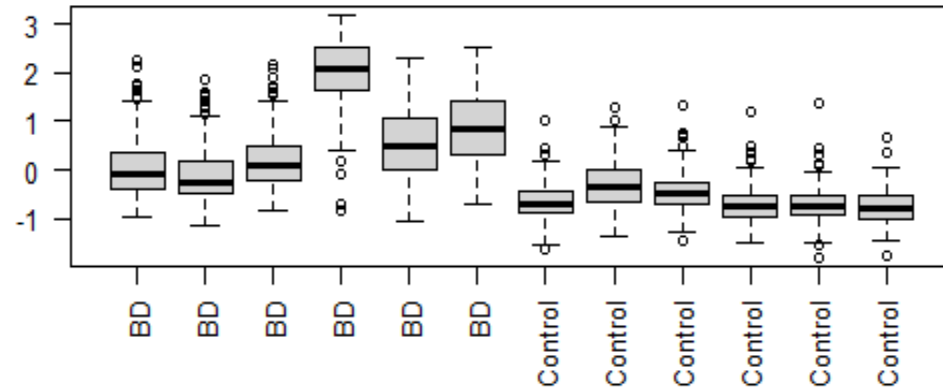
# Identification of the Co-expression Modules

- Goal: identify smaller gene clusters, without specifying their number
- Weighted Gene Co-expression Network Analysis (WGCNA)
  - 499 out of 549 genes were assigned to a module
  - A total of 4 modules were identified

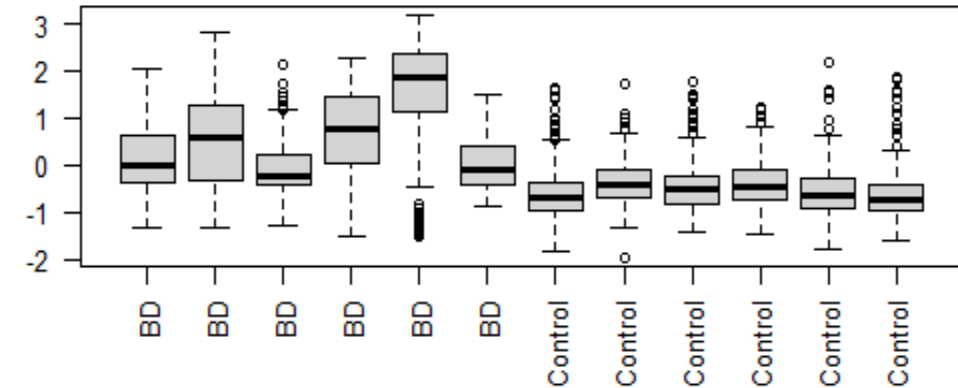


# Modules display specific expression patterns for BD patients

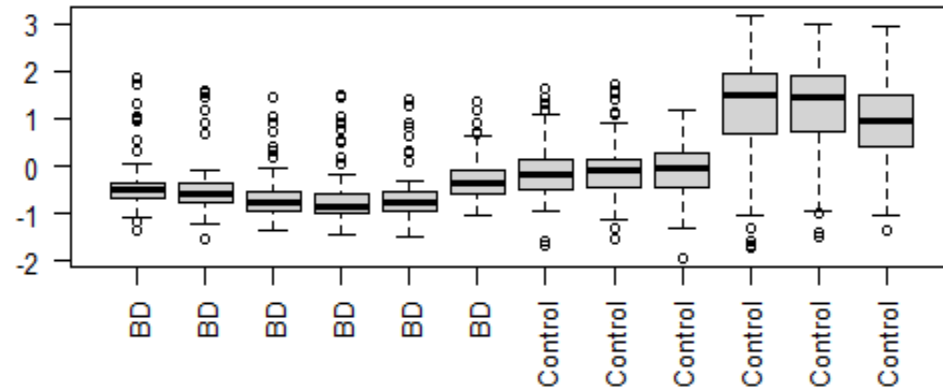
Module 1 (191)



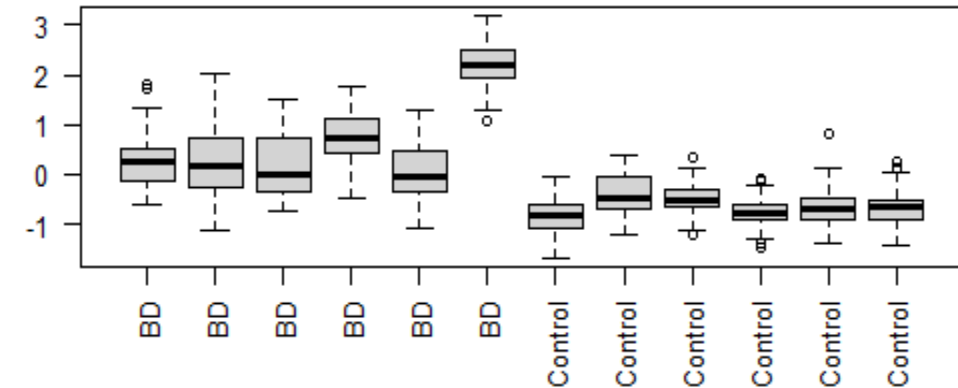
Module 2 (164)



Module 3 (97)



Module 4 (47)



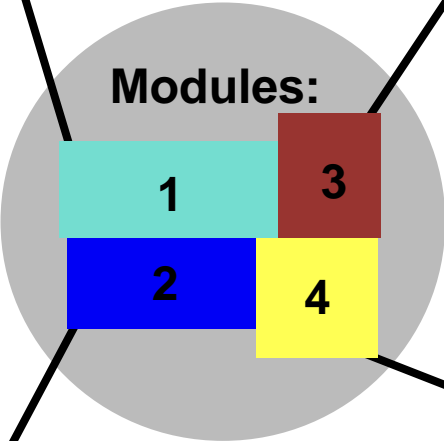
# Gene Enrichment Analysis – Frequency Comparison DAVID

Category	Term	RT	Genes	Count	%	P-Value	Benjamini	FDR
GOTERM_CC_DIRECT	integral component of membrane	RT		20	16.4	5.7E-2	1.0E0	1.0E0
UP_SEQ_FEATURE	TRANSMEM:Helical	RT		20	16.4	7.4E-2	1.0E0	1.0E0
UP_KW_PTM	Glycoprotein	RT		16	13.1	8.1E-2	4.9E-1	4.9E-1
UP_KW_PTM	Disulfide bond	RT		15	12.3	3.0E-2	3.6E-1	3.6E-1
UP_SEQ_FEATURE	TOPO_DOM:Cytoplasmic	RT		15	12.3	9.9E-2	1.0E0	1.0E0
UP_SEQ_FEATURE	TOPO_DOM:Extracellular	RT		14	11.5	3.2E-2	7.5E-1	7.5E-1
UP_KW_DISEASE	Disease variant	RT		12	9.8	8.7E-2	9.6E-1	9.6E-1
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=7	RT		10	8.2	5.9E-4	3.8E-2	3.8E-2
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=6	RT		10	8.2	1.0E-3	3.8E-2	3.8E-2
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=5	RT		10	8.2	1.1E-3	3.8E-2	3.8E-2
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=3	RT		10	8.2	1.2E-3	3.8E-2	3.8E-2
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=4	RT		10	8.2	1.2E-3	3.8E-2	3.8E-2
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=1	RT		10	8.2	1.4E-3	3.8E-2	3.8E-2
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=2	RT		10	8.2	1.4E-3	3.8E-2	3.8E-2
UP_KW_MOLECULAR_FUNCTION	Receptor	RT		10	8.2	6.9E-2	3.3E-1	3.0E-1
GOTERM_CC_DIRECT	extracellular exosome	RT		10	8.2	9.3E-2	1.0E0	1.0E0
UP_SEQ_FEATURE	DOMAIN:G_PROTEIN_RECEP_F1_2	RT		9	7.4	1.4E-4	3.1E-2	3.1E-2
INTERPRO	G protein-coupled receptor, rhodopsin-like	RT		9	7.4	5.7E-4	2.8E-2	2.8E-2
INTERPRO	GPCR, rhodopsin-like, 7TM	RT		9	7.4	6.7E-4	2.8E-2	2.8E-2
GOTERM_BP_DIRECT	G-protein coupled receptor signaling pathway	RT		9	7.4	2.4E-3	4.0E-1	4.0E-1
UP_KW_MOLECULAR_FUNCTION	G-protein coupled receptor	RT		9	7.4	2.5E-3	4.9E-2	4.5E-2
UP_KW_MOLECULAR_FUNCTION	Transducer	RT		9	7.4	4.1E-3	4.9E-2	4.5E-2
GOTERM_BP_DIRECT	detection of chemical stimulus involved in sensory perception of smell	RT		8	6.6	1.0E-4	3.3E-2	3.3E-2
INTERPRO	Olfactory receptor	RT		8	6.6	1.4E-4	1.8E-2	1.8E-2
GOTERM_MF_DIRECT	olfactory receptor activity	RT		8	6.6	1.7E-4	1.8E-2	1.8E-2
UP_KW_BIOLOGICAL_PROCESS	Olfaction	RT		8	6.6	3.2E-4	1.1E-2	1.1E-2

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
<input type="checkbox"/>	UP_KW_CELLULAR_COMPONENT	Membrane	RT		45	58.4	1.0E-6	7.4E-6
<input type="checkbox"/>	GOTERM_MF_DIRECT	protein binding	RT		45	58.4	5.5E-6	6.4E-1
<input type="checkbox"/>	GOTERM_CC_DIRECT	plasma membrane	RT		38	49.4	1.3E-9	9.2E-8
<input type="checkbox"/>	UP_SEQ_FEATURE	CARBOHYD:N-linked (GlcNAc...) asparagine	RT		36	46.8	8.5E-10	3.6E-7
<input type="checkbox"/>	UP_KW_PTM	Glycoprotein	RT		36	46.8	2.5E-6	2.1E-5
<input type="checkbox"/>	UP_KW_DOMAIN	Transmembrane helix	RT		36	46.8	3.6E-5	3.7E-4
<input type="checkbox"/>	UP_KW_DOMAIN	Transmembrane	RT		36	46.8	4.9E-5	3.7E-4
<input type="checkbox"/>	UP_SEQ_FEATURE	TRANSMEM:Helical	RT		35	45.5	8.3E-7	7.0E-5
<input type="checkbox"/>	GOTERM_CC_DIRECT	integral component of membrane	RT		34	44.2	2.3E-6	4.7E-5
<input type="checkbox"/>	UP_KW_PTM	Disulfide bond	RT		33	42.9	5.9E-7	9.9E-6
<input type="checkbox"/>	UP_SEQ_FEATURE	TOPO_DOM:Cytoplasmic	RT		30	39.0	2.2E-7	2.3E-5
<input type="checkbox"/>	UP_KW_CELLULAR_COMPONENT	Cell membrane	RT		30	39.0	8.7E-7	7.4E-6
<input type="checkbox"/>	UP_KW_DOMAIN	Signal	RT		29	37.7	1.8E-4	9.2E-4
<input type="checkbox"/>	UP_SEQ_FEATURE	TOPO_DOM:Extracellular	RT		28	36.4	1.4E-8	2.9E-6
<input type="checkbox"/>	UP_KW_BIOLOGICAL_PROCESS	Immunity	RT		19	24.7	5.7E-10	2.2E-8
<input type="checkbox"/>	UP_KW_MOLECULAR_FUNCTION	Receptor	RT		18	23.4	3.9E-5	1.4E-3
<input type="checkbox"/>	GOTERM_CC_DIRECT	extracellular exosome	RT		17	22.1	4.7E-4	4.4E-3
<input type="checkbox"/>	GOTERM_CC_DIRECT	integral component of plasma membrane	RT		16	20.8	2.0E-5	2.9E-4

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
UP_KW_DOMAIN	Transmembrane helix	RT		24	27.0	2.0E-3	1.4E-2
UP_KW_DOMAIN	Transmembrane	RT		24	27.0	2.4E-3	1.4E-2
UP_SEQ_FEATURE	TRANSMEM:Helical	RT		22	24.7	7.8E-3	4.8E-1
GOTERM_CC_DIRECT	integral component of membrane	RT		21	23.6	1.5E-2	8.6E-1
UP_KW_PTM	Glycoprotein	RT		18	20.2	7.7E-3	6.1E-2
GOTERM_CC_DIRECT	plasma membrane	RT		18	20.2	6.3E-2	8.6E-1
UP_KW_PTM	Disulfide bond	RT		16	18.0	5.9E-3	6.1E-2
UP_SEQ_FEATURE	CARBOHYD:N-linked (GlcNAc...) asparagine	RT		16	18.0	8.1E-2	1.0E0
UP_KW_CELLULAR_COMPONENT	Cell membrane	RT		15	16.9	5.8E-2	9.7E-1
UP_KW_BIOLOGICAL_PROCESS	Transport	RT		14	15.7	3.5E-3	1.1E-1
UP_SEQ_FEATURE	TOPO_DOM:Extracellular	RT		12	13.5	8.1E-2	1.0E0
GOTERM_BP_DIRECT	positive regulation of cell proliferation	RT		5	5.6	3.3E-2	1.0E0
UP_SEQ_FEATURE	DOMAIN:G_PROTEIN_RECEP_F1_2	RT		5	5.6	5.5E-2	9.3E-1
GOTERM_BP_DIRECT	response to hydrogen peroxide	RT		4	4.5	2.2E-4	7.8E-2
UP_KW_MOLECULAR_FUNCTION	Translocase	RT		4	4.5	2.2E-3	5.2E-2
GOTERM_BP_DIRECT	heart development	RT		4	4.5	1.3E-2	1.0E0
GOTERM_CC_DIRECT	apical plasma membrane	RT		4	4.5	5.7E-2	8.6E-1
UP_KW_LIGAND	Iron	RT		4	4.5	6.0E-2	4.0E-1
GOTERM_CC_DIRECT	external side of plasma membrane	RT		4	4.5	9.0E-2	9.6E-1
UP_KW_BIOLOGICAL_PROCESS	Electron transport	RT		3	3.4	4.1E-2	3.4E-1
GOTERM_MF_DIRECT	heme binding	RT		3	3.4	5.0E-2	1.0E0
UP_KW_LIGAND	Heme	RT		3	3.4	5.1E-2	4.0E-1
GOTERM_BP_DIRECT	positive regulation of angiogenesis	RT		3	3.4	5.2E-2	1.0E0
UP_KW_LIGAND	NAD	RT		3	3.4	9.4E-2	4.0E-1
OMIM_DISEASE	Erythrocytosis 7	RT		2	2.2	4.1E-3	1.1E-1
UP_SEQ_FEATURE	PEPTIDE:Hemopressin	RT		2	2.2	4.8E-3	4.8E-1

Category	Term	RT	Genes	Count	%	P-Value	Benjamini
GOTERM_CC_DIRECT	cytoskeleton	RT		4	15.4	2.6E-3	1.1E-1
UP_KW_BIOLOGICAL_PROCESS	Transport	RT		4	15.4	8.5E-2	4.7E-1
UP_KW_BIOLOGICAL_PROCESS	Ion transport	RT		3	11.5	4.4E-2	4.7E-1
GOTERM_BP_DIRECT	cellular iron ion homeostasis	RT		2	7.7	2.7E-2	1.0E0
GOTERM_CC_DIRECT	microvillus	RT		2	7.7	3.8E-2	8.3E-1
GOTERM_BP_DIRECT	epidermis development	RT		2	7.7	3.8E-2	1.0E0
UP_KW_CELLULAR_COMPONENT	Flagellum	RT		2	7.7	7.8E-2	9.4E-1





# Gene Enrichment Analysis – Frequency Comparison DAVID

Category	Term
GOTERM_CC_DIRECT	integral component of membrane
UP_SEQ_FEATURE	TRANSMEM:Helical
UP_KW_PT	Glycoprotein
UP_KW_PT	Disulfide bond
UP_SEQ_FEATURE	TOPO_DOM:Cytoplasmic
UP_SEQ_FEATURE	TOPO_DOM:Extracellular
UP_KW_DISEASE	Disease variant
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=7
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=6
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=5
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=3
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=4
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=1
UP_SEQ_FEATURE	TRANSMEM:Helical; Name=2
UP_KW_MOLECULAR_FUNCTION	Receptor
GOTERM_CC_DIRECT	extracellular exosome
UP_SEQ_FEATURE	DOMAIN:G_PROTEIN_RECEP_F1_2
INTERPRO	G protein-coupled receptor, rhodopsin-like
INTERPRO	GPCR, rhodopsin-like, 7TM
GOTERM_BP_DIRECT	G-protein coupled receptor signaling pathway
UP_KW_MOLECULAR_FUNCTION	G-protein coupled receptor
UP_KW_MOLECULAR_FUNCTION	Transducer
GOTERM_BP_DIRECT	detection of chemical stimulus involved in sensory perception of smell
INTERPRO	Olfactory receptor
GOTERM_MF_DIRECT	olfactory receptor activity
UP_KW_BIOLOGICAL_PROCESS	Olfaction

Membrane Protein  
GPCR

Membrane Protein  
Glycosylation

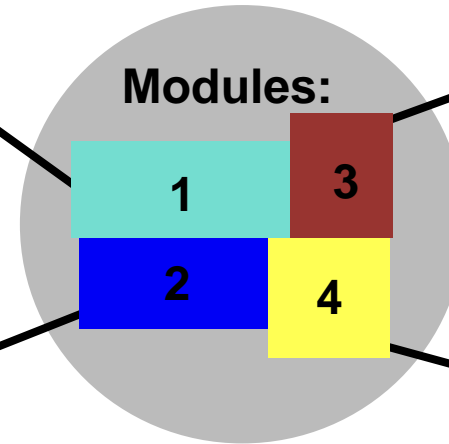
Category	Term
UP_KW_CELLULAR_COMPONENT	Membrane
GOTERM_MF_DIRECT	protein binding
GOTERM_CC_DIRECT	plasma membrane
UP_SEQ_FEATURE	CARBOHYD:N-linked (GlcNAc...) asparagine
UP_KW_PT	Glycoprotein
UP_KW_DOMAIN	Transmembrane helix
UP_KW_DOMAIN	Transmembrane
UP_SEQ_FEATURE	TRANSMEM:Helical
GOTERM_CC_DIRECT	integral component of membrane
UP_KW_PT	Disulfide bond
UP_SEQ_FEATURE	TOPO_DOM:Cytoplasmic
UP_KW_CELLULAR_COMPONENT	Cell membrane
UP_KW_DOMAIN	Signal
UP_SEQ_FEATURE	TOPO_DOM:Extracellular
UP_KW_BIOLOGICAL_PROCESS	Immunity
UP_KW_MOLECULAR_FUNCTION	Receptor
GOTERM_CC_DIRECT	extracellular exosome
GOTERM_CC_DIRECT	integral component of plasma membrane

Category	Term
UP_KW_DOMAIN	Transmembrane helix
UP_KW_DOMAIN	Transmembrane
UP_SEQ_FEATURE	TRANSMEM:Helical
GOTERM_CC_DIRECT	integral component of membrane
UP_KW_PT	Glycoprotein
GOTERM_CC_DIRECT	plasma membrane
UP_KW_PT	Disulfide bond
UP_SEQ_FEATURE	CARBOHYD:N-linked (GlcNAc...) asparagine
UP_KW_CELLULAR_COMPONENT	Cell membrane
UP_KW_BIOLOGICAL_PROCESS	Transport
UP_SEQ_FEATURE	TOPO_DOM:Extracellular
GOTERM_BP_DIRECT	positive regulation of cell proliferation
UP_SEQ_FEATURE	DOMAIN:G_PROTEIN_RECEP_F1_2
GOTERM_BP_DIRECT	response to hydrogen peroxide
UP_KW_MOLECULAR_FUNCTION	Translocase
GOTERM_BP_DIRECT	heart development
GOTERM_CC_DIRECT	apical plasma membrane
UP_KW_LIGAND	Iron
GOTERM_CC_DIRECT	external side of plasma membrane
UP_KW_BIOLOGICAL_PROCESS	Electron transport
GOTERM_MF_DIRECT	heme binding
UP_KW_LIGAND	Heme
GOTERM_BP_DIRECT	positive regulation of angiogenesis
UP_KW_LIGAND	NAD
OMIM_DISEASE	Erythrocytosis 7
UP_SEQ_FEATURE	PEPTIDE:Hemopressin

Membrane Protein  
Cardiac / Developmental  
RedOx / Metallo Protein

Cell Appendages / Structures  
Ion Transport

Category	Term
GOTERM_CC_DIRECT	cytoskeleton
UP_KW_BIOLOGICAL_PROCESS	Transport
UP_KW_BIOLOGICAL_PROCESS	Ion transport
GOTERM_BP_DIRECT	cellular iron ion homeostasis
GOTERM_CC_DIRECT	microvillus
GOTERM_BP_DIRECT	epidermis development
UP_KW_CELLULAR_COMPONENT	Flagellum



# Gene Enrichment Analysis – Rank Distribution Comparison GSEA

- **Gene Set Enrichment Analysis (GSEA):** rank-based statistical tests for enrichment analysis
- Advantages vs DAVID:
  - No arbitrary threshold for DE, gene list of interest
  - Genes ordered based on:  $s_g = \text{sign}(\log FC_g) \times (-\log_{10} P_g)$
- Estimated significance is created with Kolmogorov-Smirnov test
- Compare normalized ES genes to curated gene subsets of Molecular Signatures Database (MSigDB)
- We chose C5: gene subsets ordered to same **Gene Ontology (GO)** term
- **Results:** GO terms that are most enriched (most genes per GO gene set)

# Gene Enrichment Analysis – GSEA (GO terms)

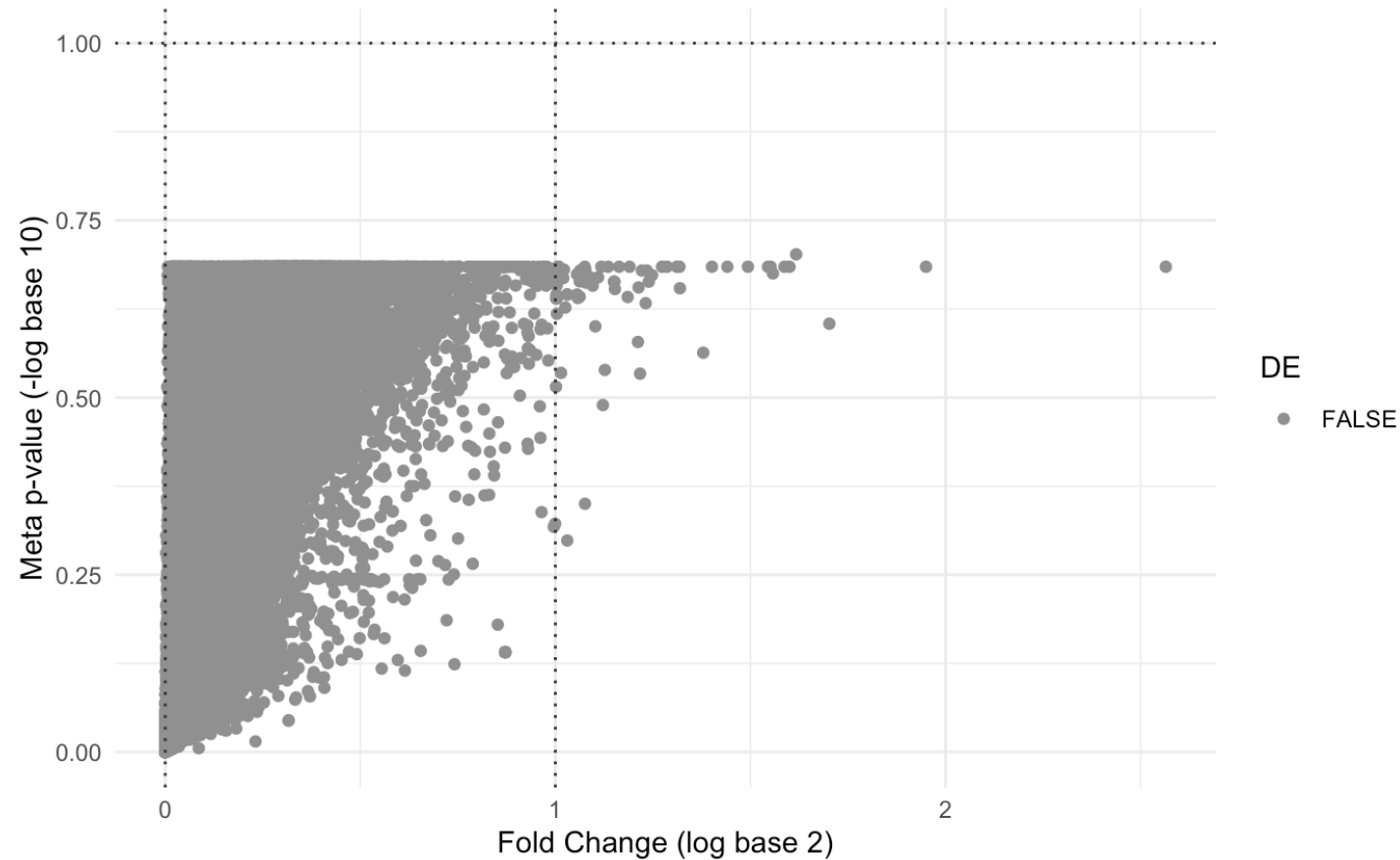
	pathway	padj	NES	size
1	GOCC_VESICLE_MEMBRANE	0.00	3.54	22
2	GOBP_HOMEOSTATIC_PROCESS	0.00	2.25	25
3	GOBP_VESICLE_MEDIATED_TRANSPORT	0.01	1.90	19
4	GOBP_CHEMICAL_HOMEOSTASIS	0.03	1.76	20
5	GOBP_ION_TRANSPORT	0.08	1.60	26
6	GOBP_REGULATION_OF_CELLULAR_COMPONENT_MOVEMENT	0.08	1.56	16
7	GOBP_REGULATION_OF_CELL_DEATH	0.08	1.53	20
8	GOMF_ION_TRANSMEMBRANE_TRANSPORTER_ACTIVITY	0.24	1.23	15
9	GOBP_POSITIVE_REGULATION_OF_CELLULAR_BIOSYNTHETIC_PROCESS	0.32	1.13	15
10	GOBP_PROGRAMMED_CELL_DEATH	0.38	1.10	19
11	GOBP_NERVOUS_SYSTEM_PROCESS	0.54	0.96	24
12	GOBP_ION_TRANSMEMBRANE_TRANSPORT	0.60	0.92	20
13	GOBP_CATION_TRANSPORT	1.00	-0.37	17

Membrane Protein/Transport  
Vesicle

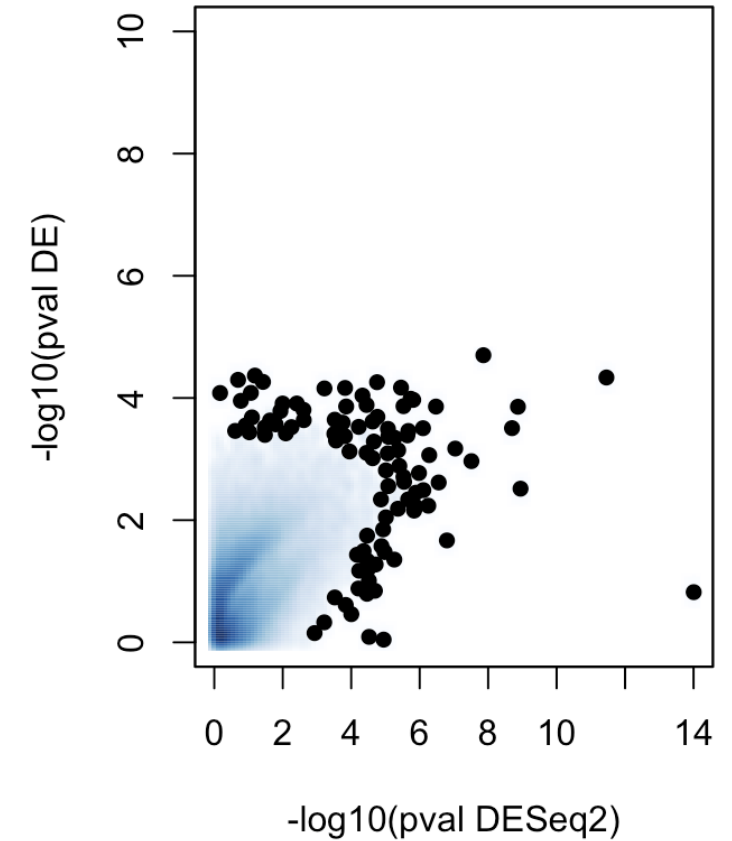
# Final Remarks

- Found 549 DE genes, 3 models with upregulated, 1 module with downregulated genes in BD
- Our pipeline allows to make comparable conclusions with Akula *et al.*, 2014
- Multiple GO terms from Akula *et al.*, 2014 were identified
  - Membrane components
  - Ion and membrane transport
  - Cell death and homeostasis
- However not all GO categories Akula *et al.*, 2014 from were confirmed
- Additionally, our analysis possibly links new aspects to bipolar disorder
  - Glycosylation
  - Cardiac, developmental and RedOx processes

## Additional: ANOVA



ANOVA results of DE analysis: fold change > 2, padj < 0.1

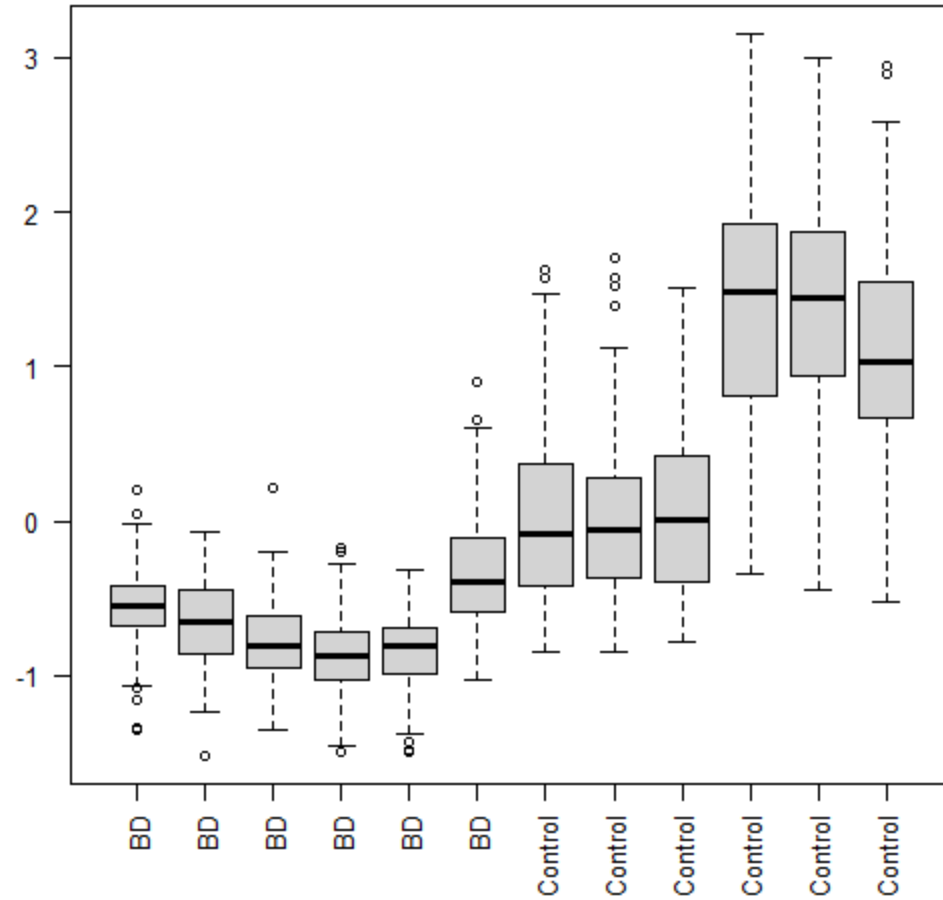


Comparison DESeq2 and ANOVA

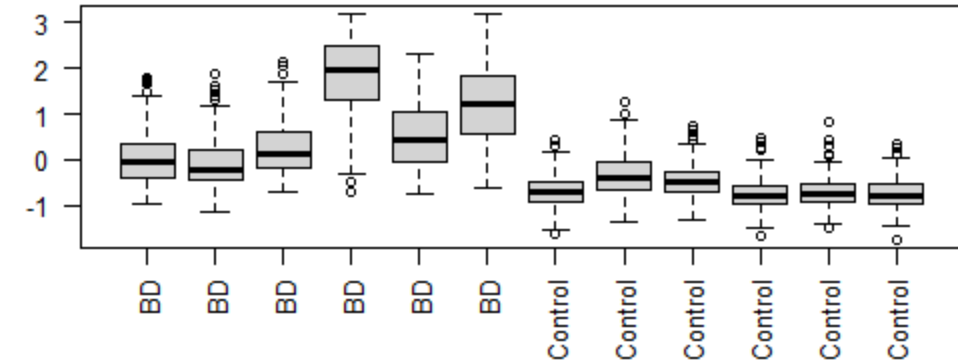


# Additional: WGCNA on Separate Clusters

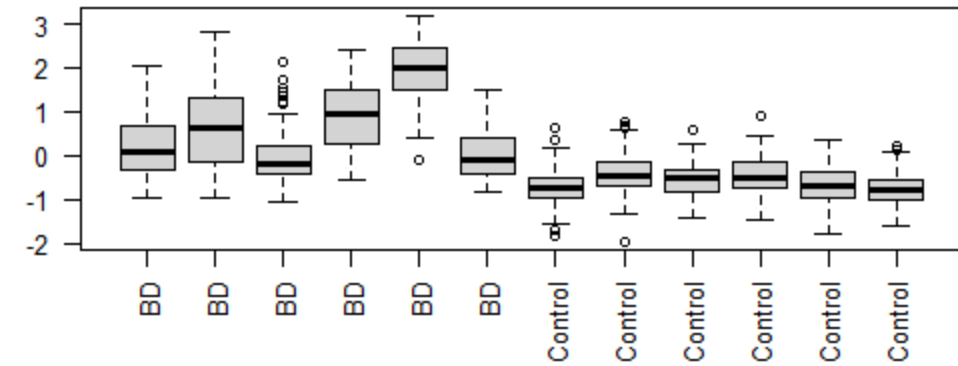
Module 1 (101)



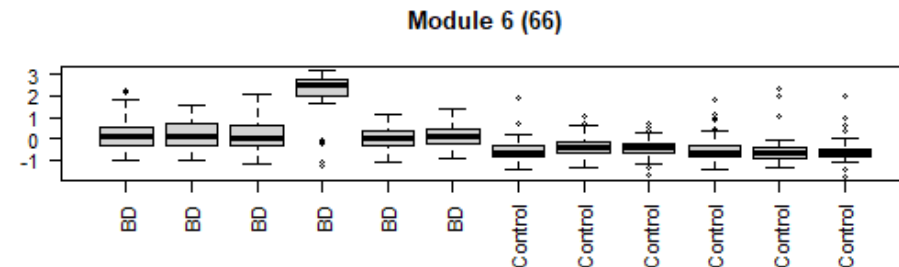
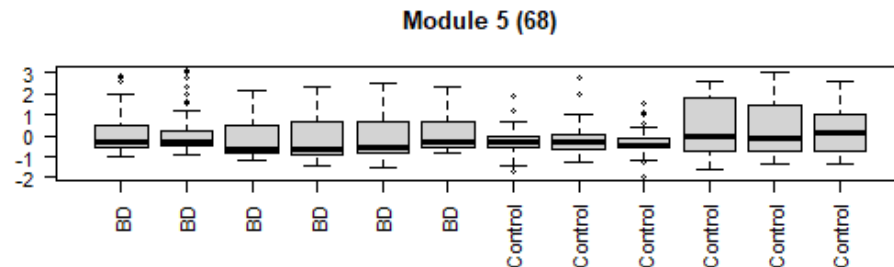
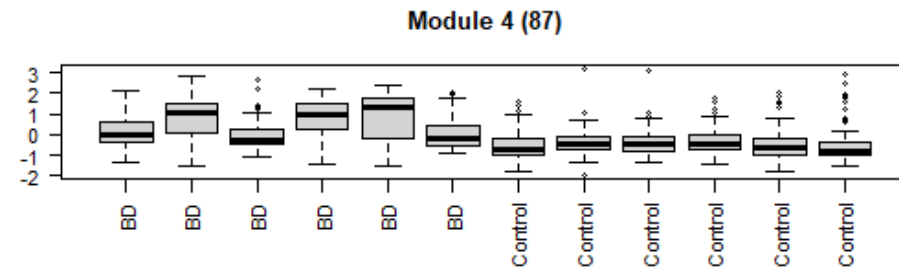
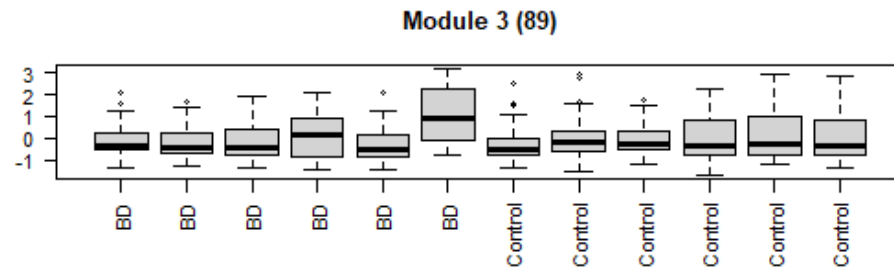
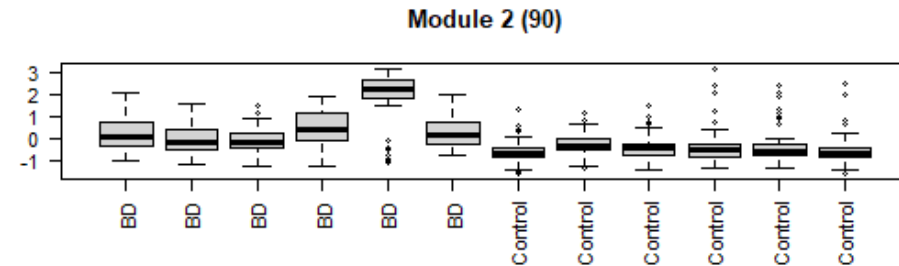
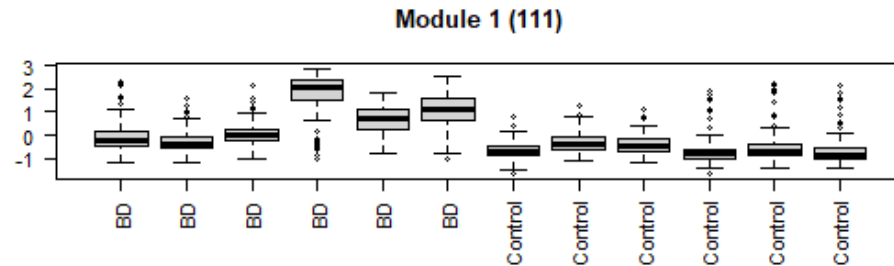
Module 1 (222)



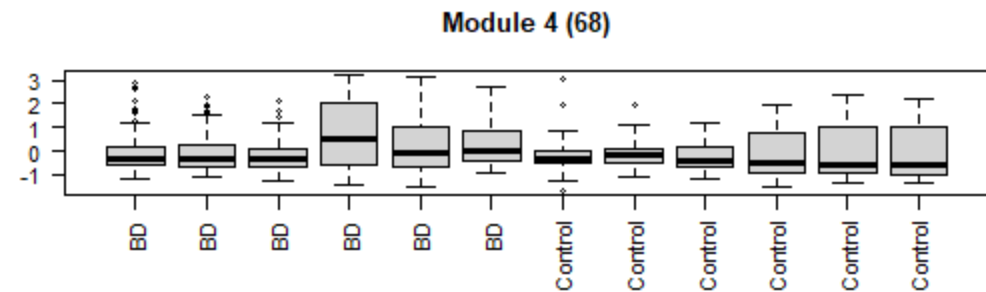
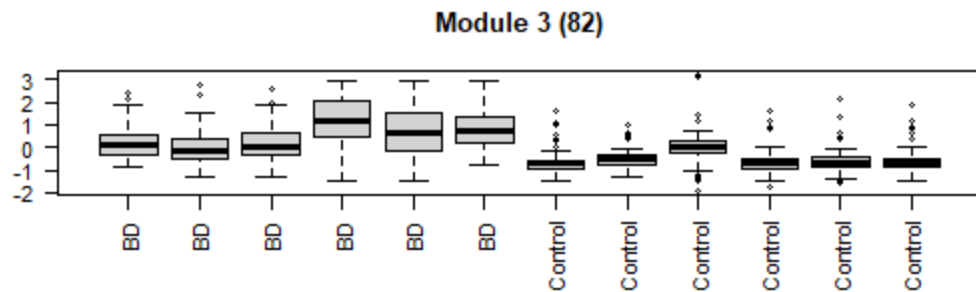
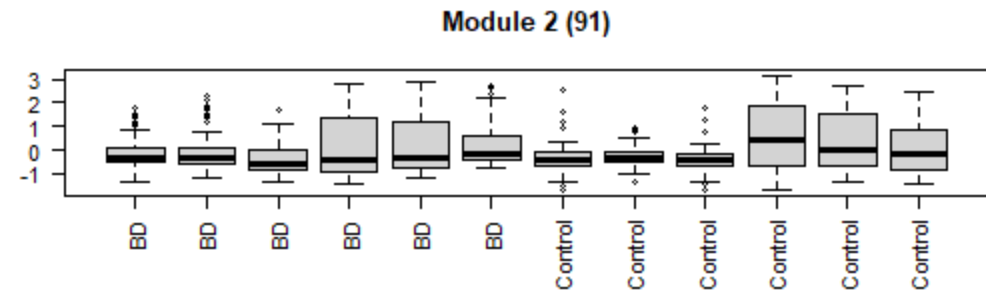
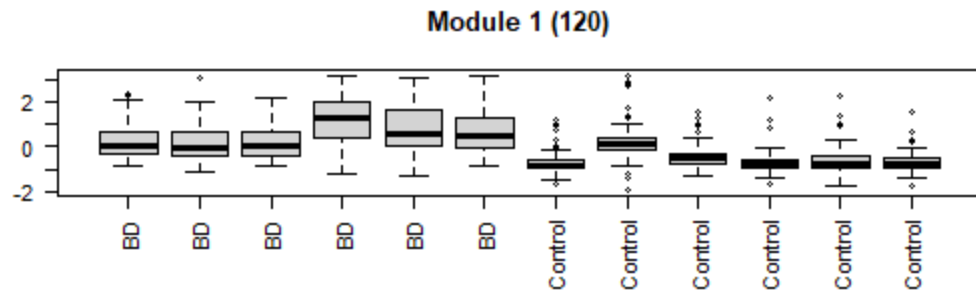
Module 2 (146)



# Additional: WGCNA on Separate Patient Groups (BD)



# Additional: WGCNA on Separate Patient Groups (Control)



# Additional: GSEA plots

