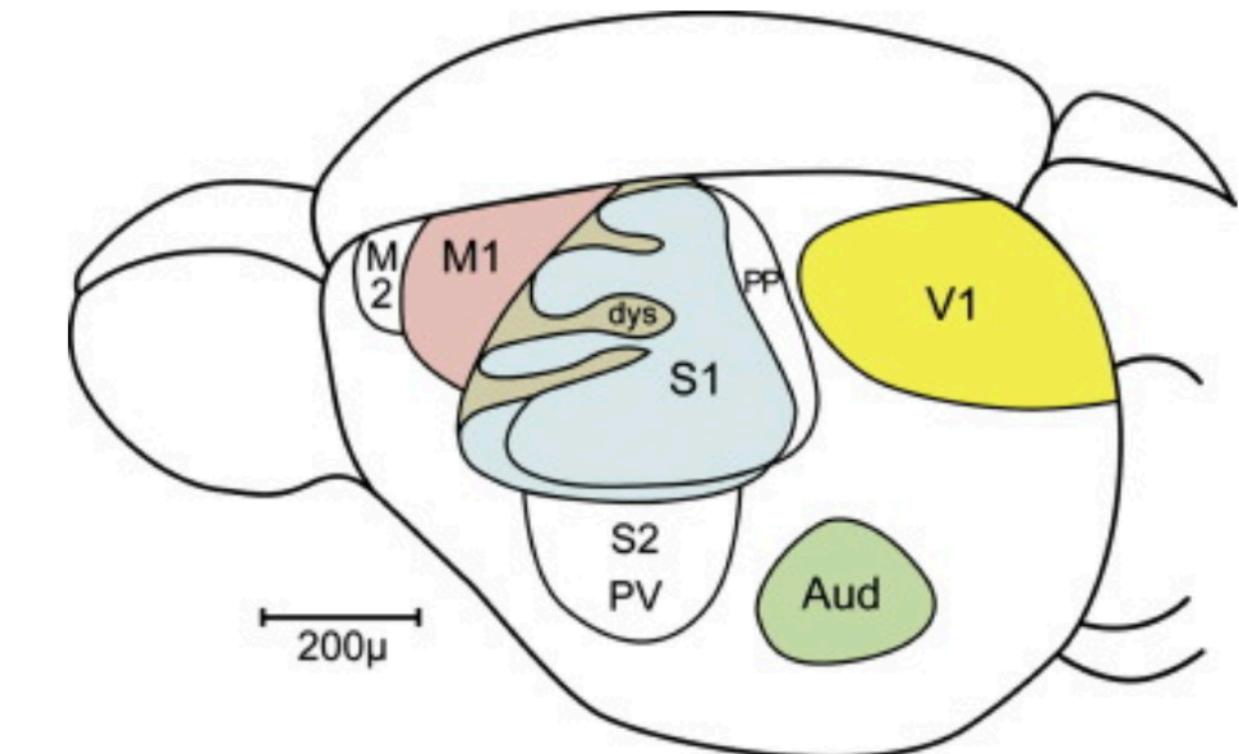
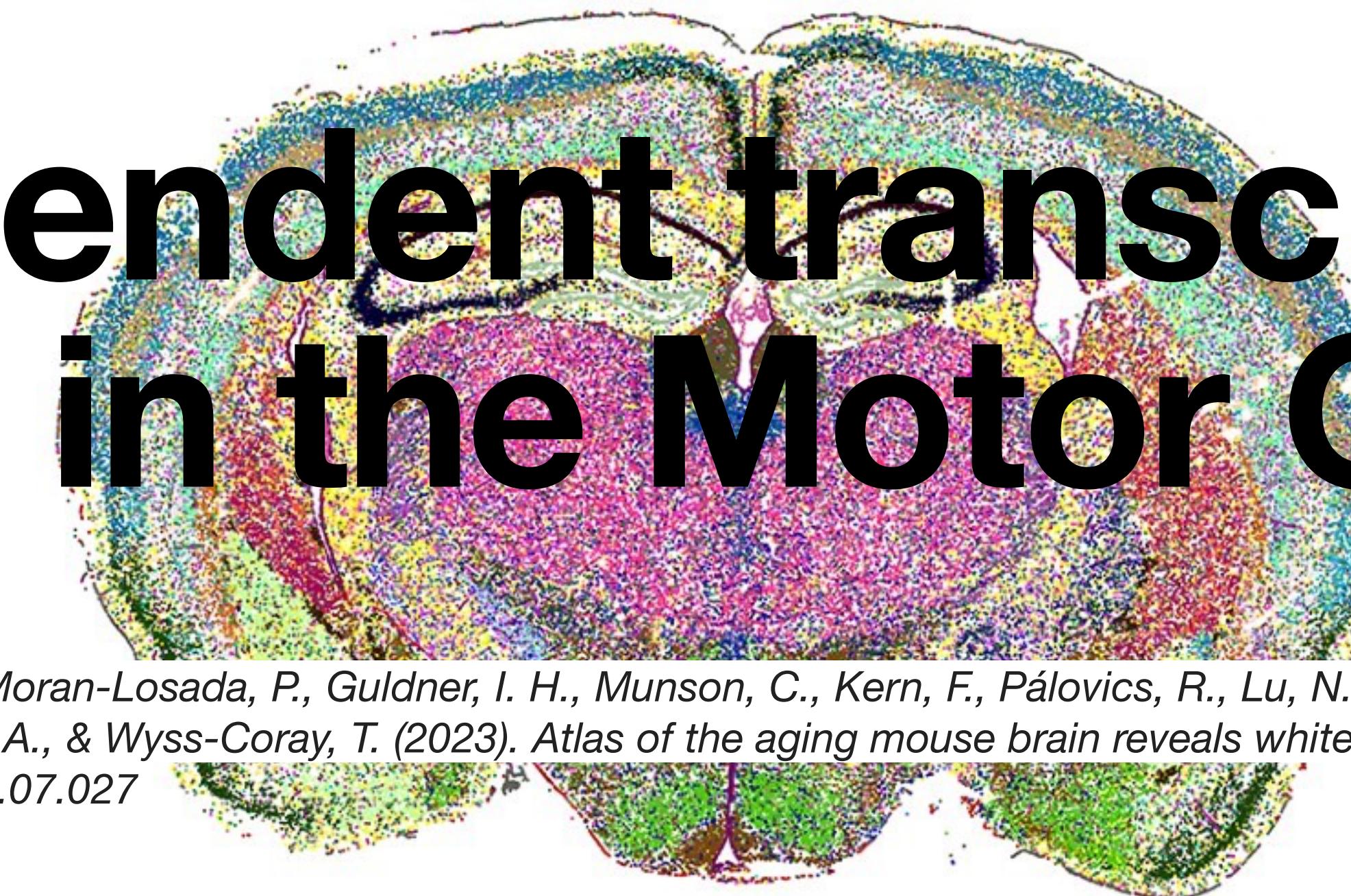
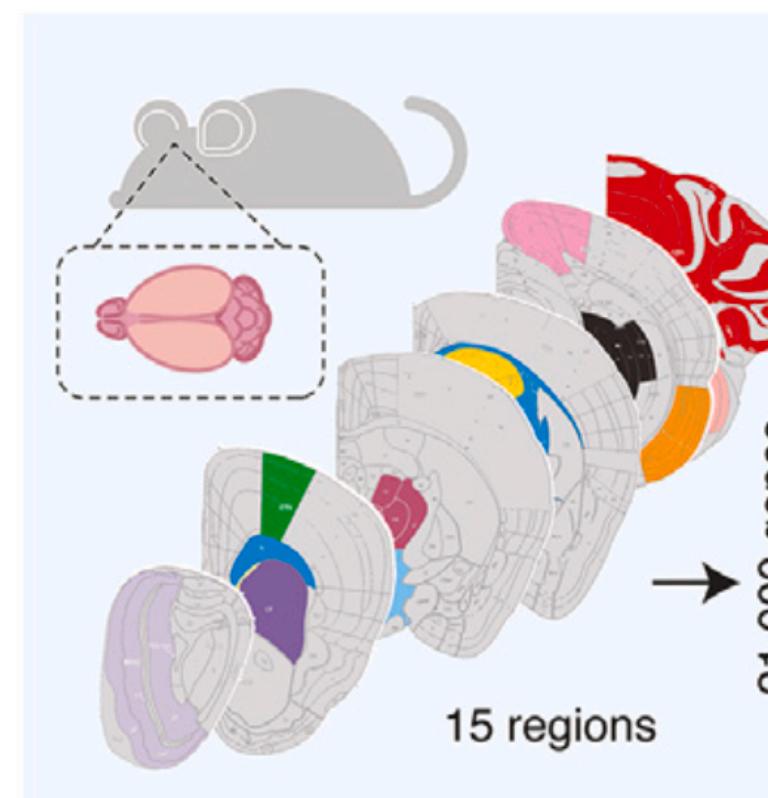


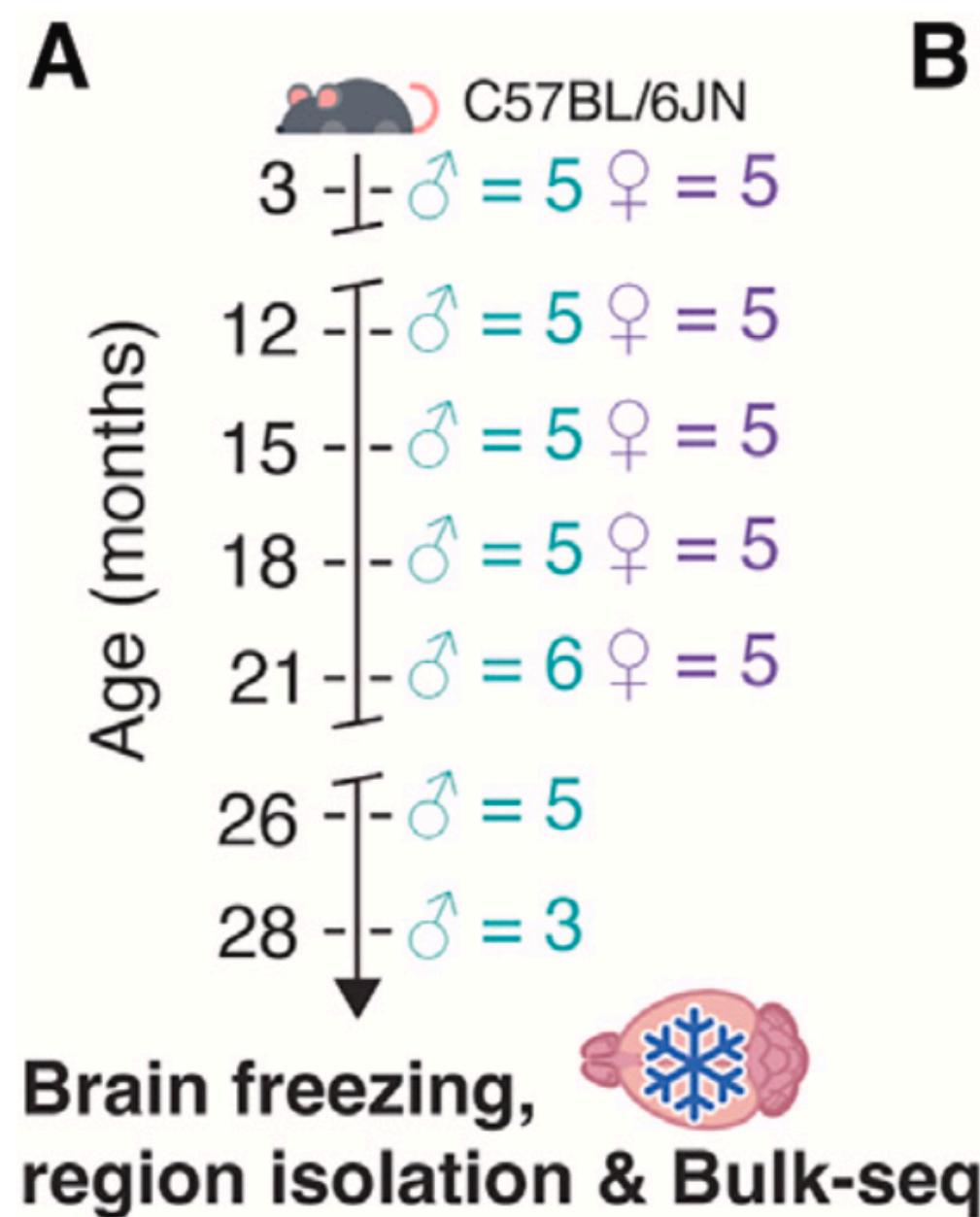
Age-dependent transcriptomic changes in the Motor Cortex

Hahn, O., Foltz, A. G., Atkins, M., Kedir, B., Moran-Losada, P., Guldner, I. H., Munson, C., Kern, F., Pálovics, R., Lu, N., Zhang, H., Kaur, A., Hull, J., Huguenard, J. R., Grönke, S., Lehallier, B., Partridge, L., Keller, A., & Wyss-Coray, T. (2023). Atlas of the aging mouse brain reveals white matter as vulnerable foci. *Cell*, 186(19), 4117–4133.e22. <https://doi.org/10.1016/j.cell.2023.07.027>



General Introduction

Paper introduction



- Spatiotemporal RNA sequencing of mouse brain
- **1076 samples from 15 regions of the brain across 7 ages**
 - 2 rejuvenation interventions
- Specialised neuronal populations showed region-specific expression changes

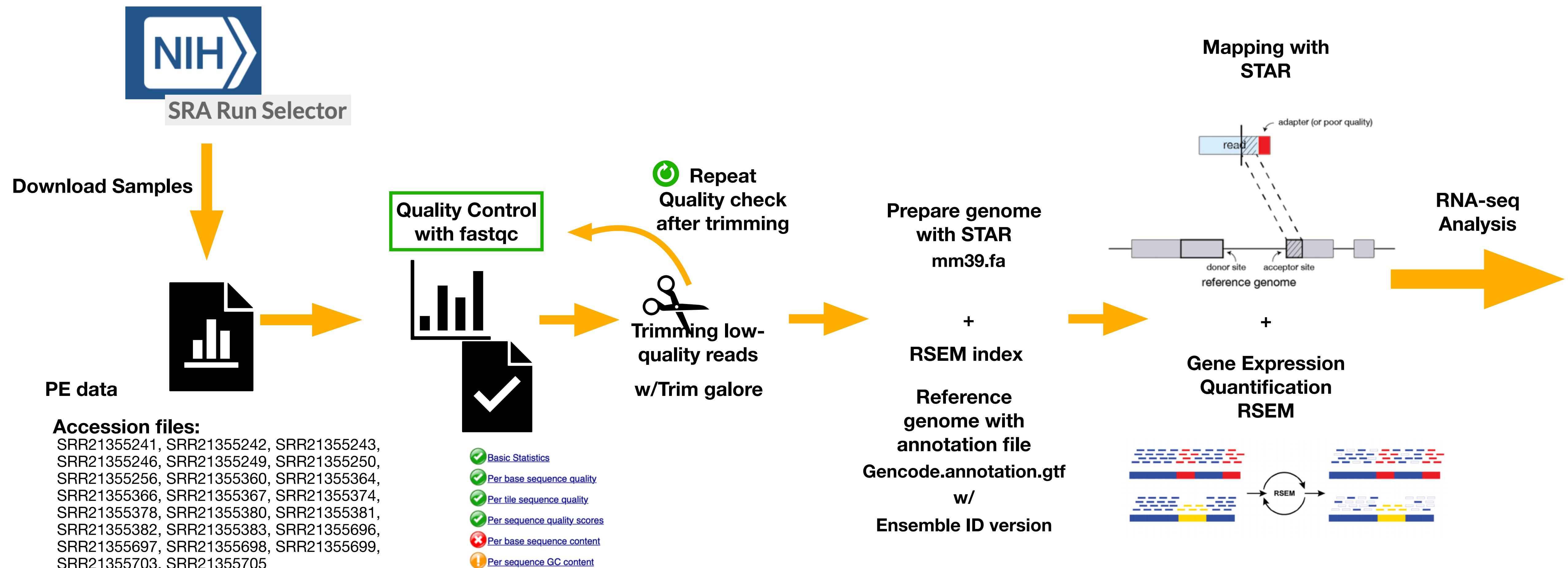
Introduction to our data analysis

Dry Lab task

- **23 samples** of the mouse brain motor cortex.
- Identify differential expression of genes over time (**age**).
- The **only covariate** in the samples is **sex**.

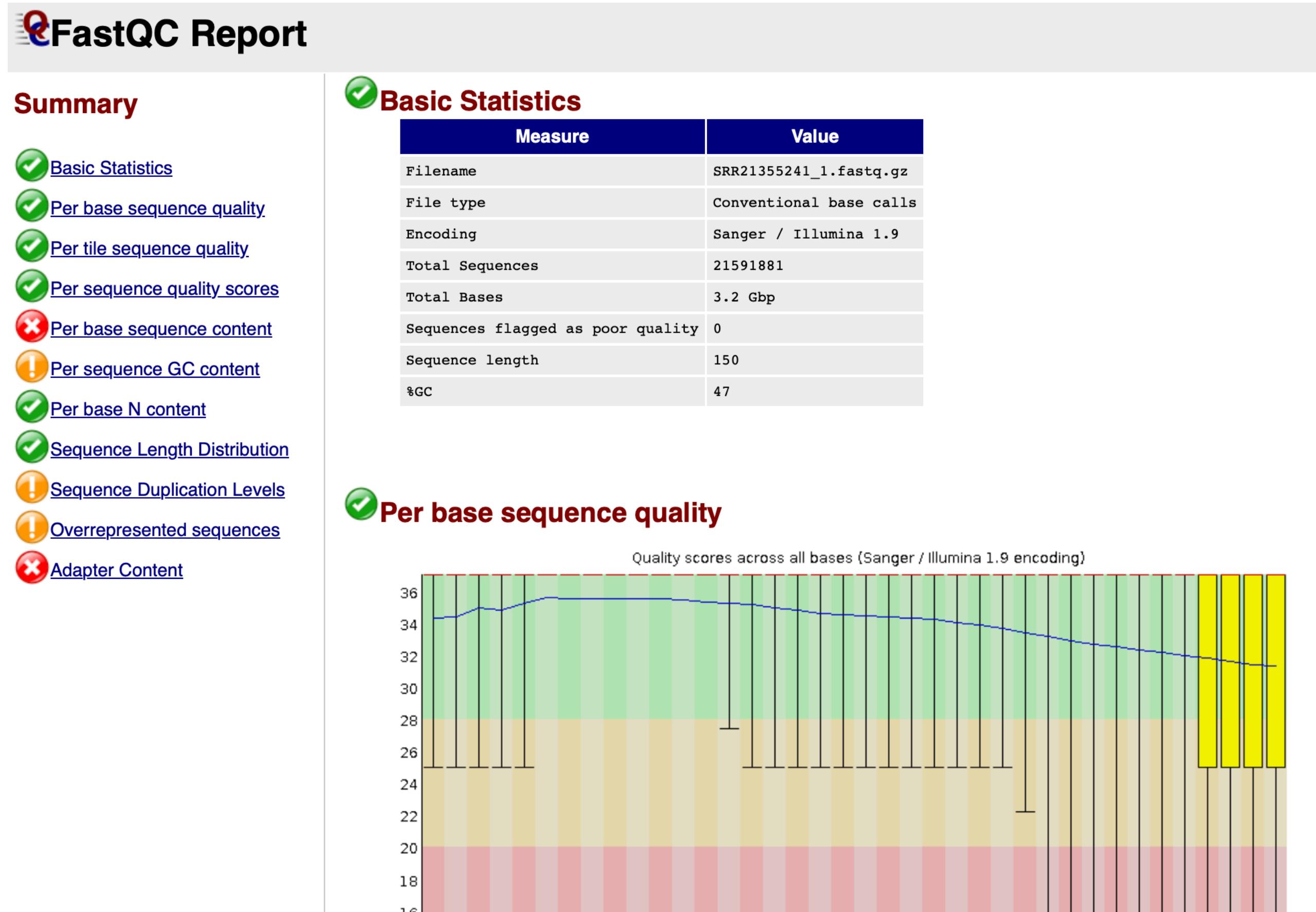
Age (in months)	3	12	15	18	21	26	28
Amount	4	5	4	4	4	1	1
Sex	2 ♀ 2 ♂	2 ♀ 3 ♂	2 ♀ 2 ♂	2 ♀ 2 ♂	2 ♀ 2 ♂	1 ♂	1 ♂

Preprocessing Pipeline



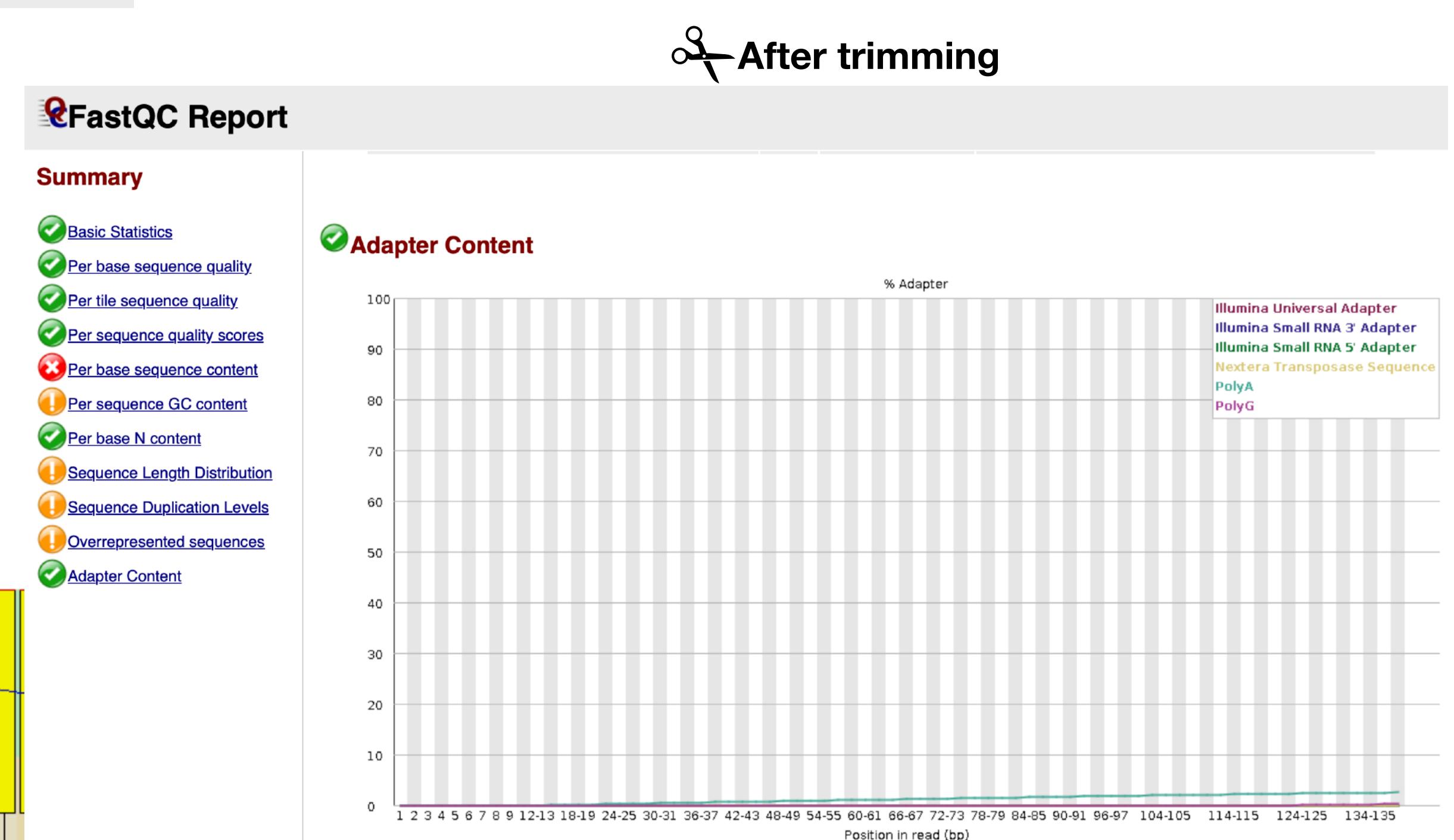
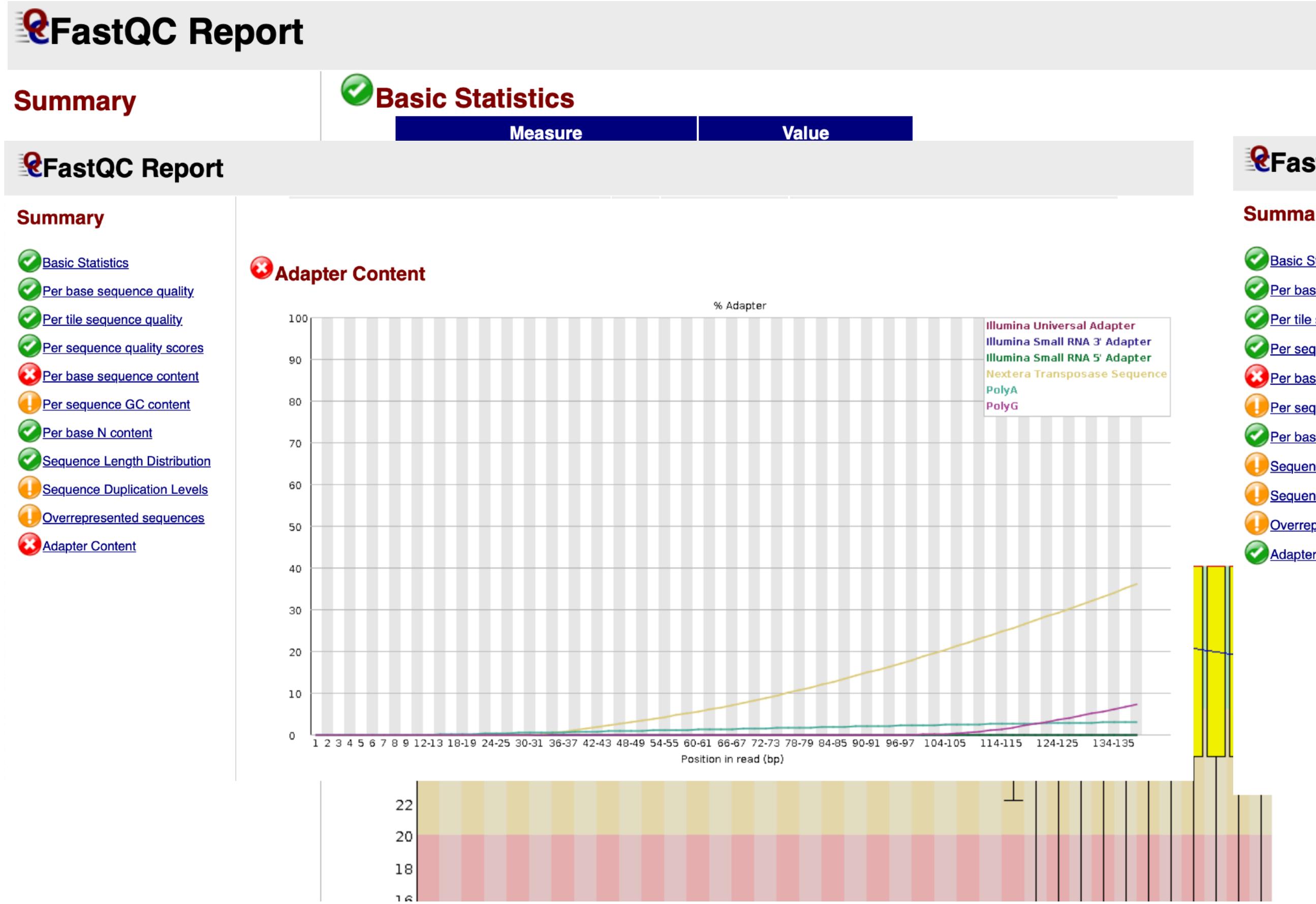
Preprocessing

Quality Control Results - Before trimming



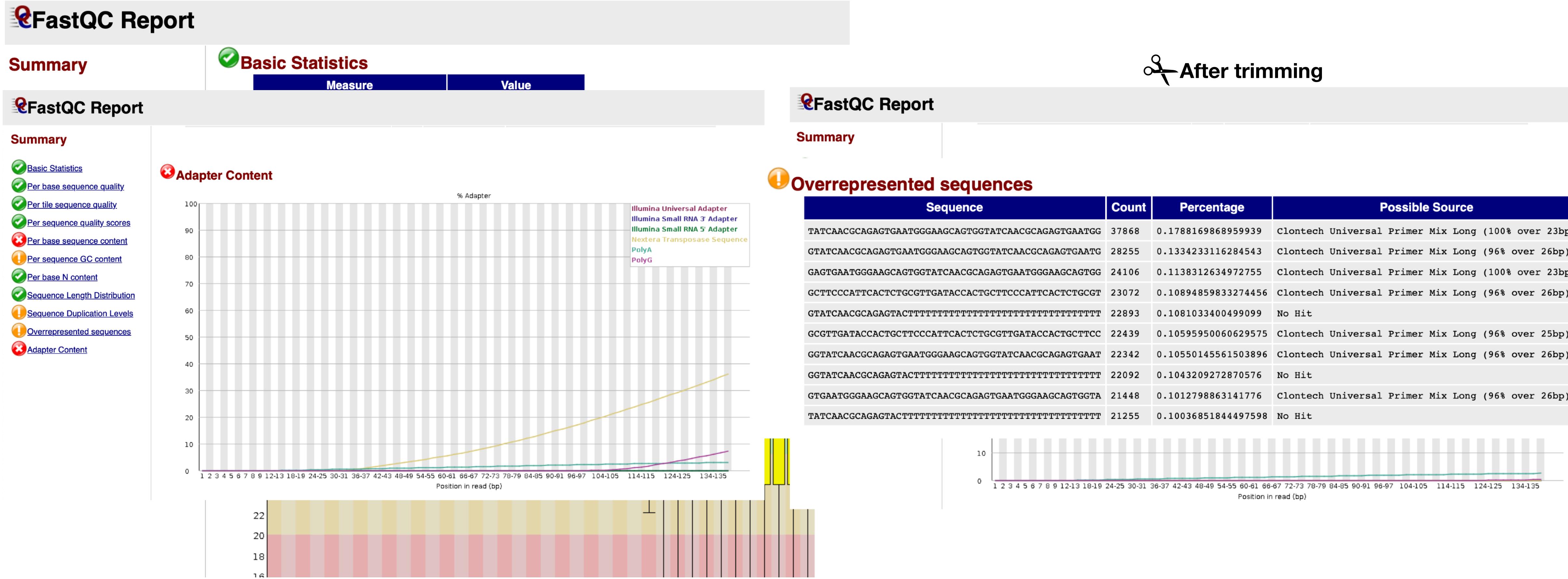
Preprocessing

Quality Control Results - Before/After trimming



Preprocessing

Quality Control Results - Before/After trimming



Preprocessing

Mapping statistics - Before/After trimming

```
Log.final.out

Started job on | Nov 09 09:09:13
Started mapping on | Nov 09 09:13:36
Finished on | Nov 09 09:25:38
Mapping speed, Million of reads per hour | 107.66

Number of input reads | 21591881
Average input read length | 300
UNIQUE READS:
    Uniquely mapped reads number | 11987521
    Uniquely mapped reads % | 55.52%
    Average mapped length | 283.41
    Number of splices: Total | 8037223
    Number of splices: Annotated (sjdb) | 7907871
    Number of splices: GT/AG | 7904572
    Number of splices: GC/AG | 61557
    Number of splices: AT/AC | 9212
    Number of splices: Non-canonical | 61882
    Mismatch rate per base, % | 0.51%
    Deletion rate per base | 0.02%
    Deletion average length | 1.89
    Insertion rate per base | 0.03%
    Insertion average length | 2.27
    MULTI-MAPPING READS:
        Number of reads mapped to multiple loci | 915508
        % of reads mapped to multiple loci | 4.24%
        Number of reads mapped to too many loci | 29291
        % of reads mapped to too many loci | 0.14%
    UNMAPPED READS:
        Number of reads unmapped: too many mismatches | 0
        % of reads unmapped: too many mismatches | 0.00%
        Number of reads unmapped: too short | 8620580
        % of reads unmapped: too short | 39.93%
        Number of reads unmapped: other | 38981
        % of reads unmapped: other | 0.18%
    CHIMERIC READS:
        Number of chimeric reads | 0
        % of chimeric reads | 0.00%
```

Uniquely mapped reads % | 55.52%

% of reads mapped to multiple loci | 4.24%

```
Log.final.out

Started job on | Nov 20 05:36:53
Started mapping on | Nov 20 05:40:55
Finished on | Nov 20 05:47:55
Mapping speed, Million of reads per hour | 181.52

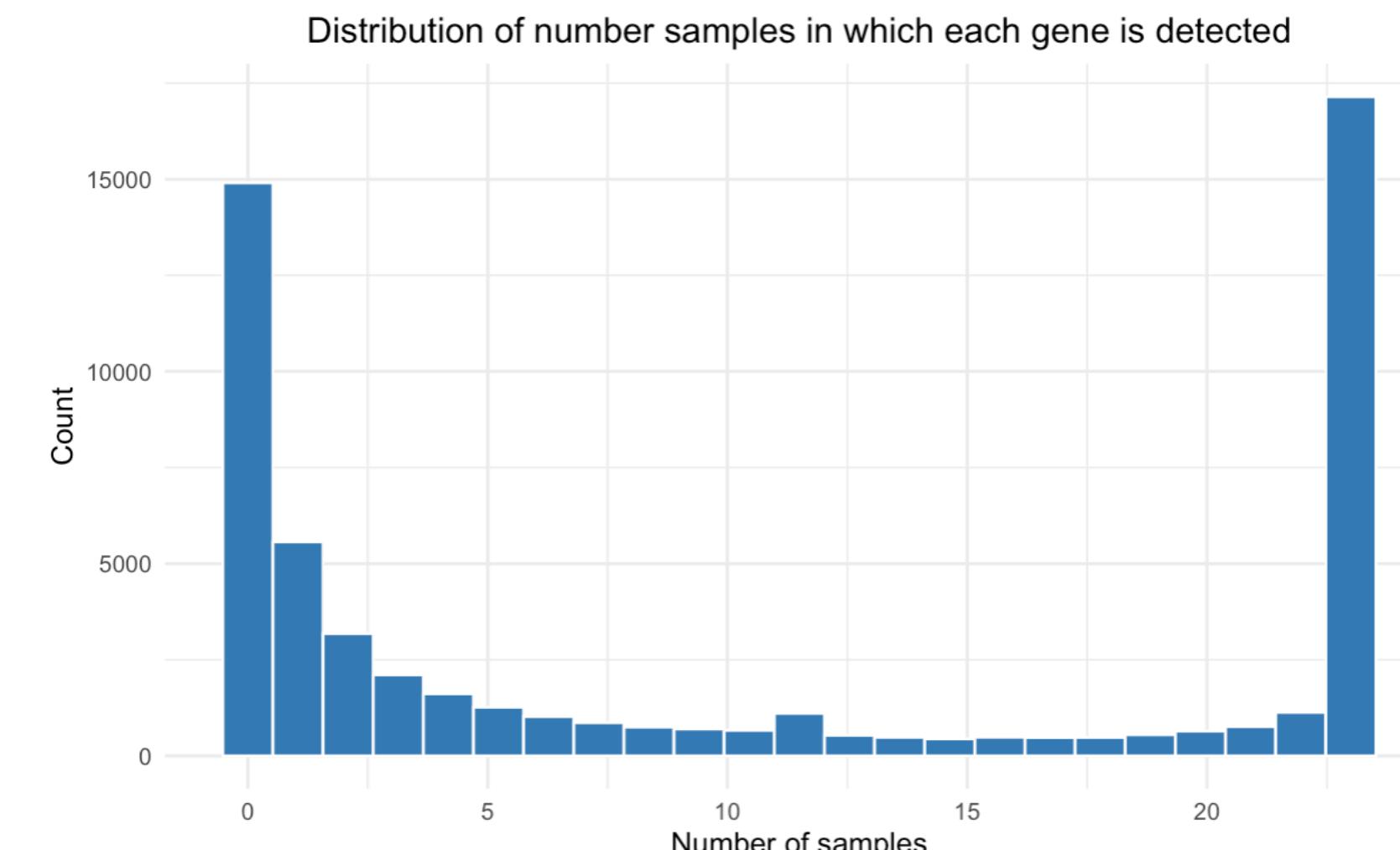
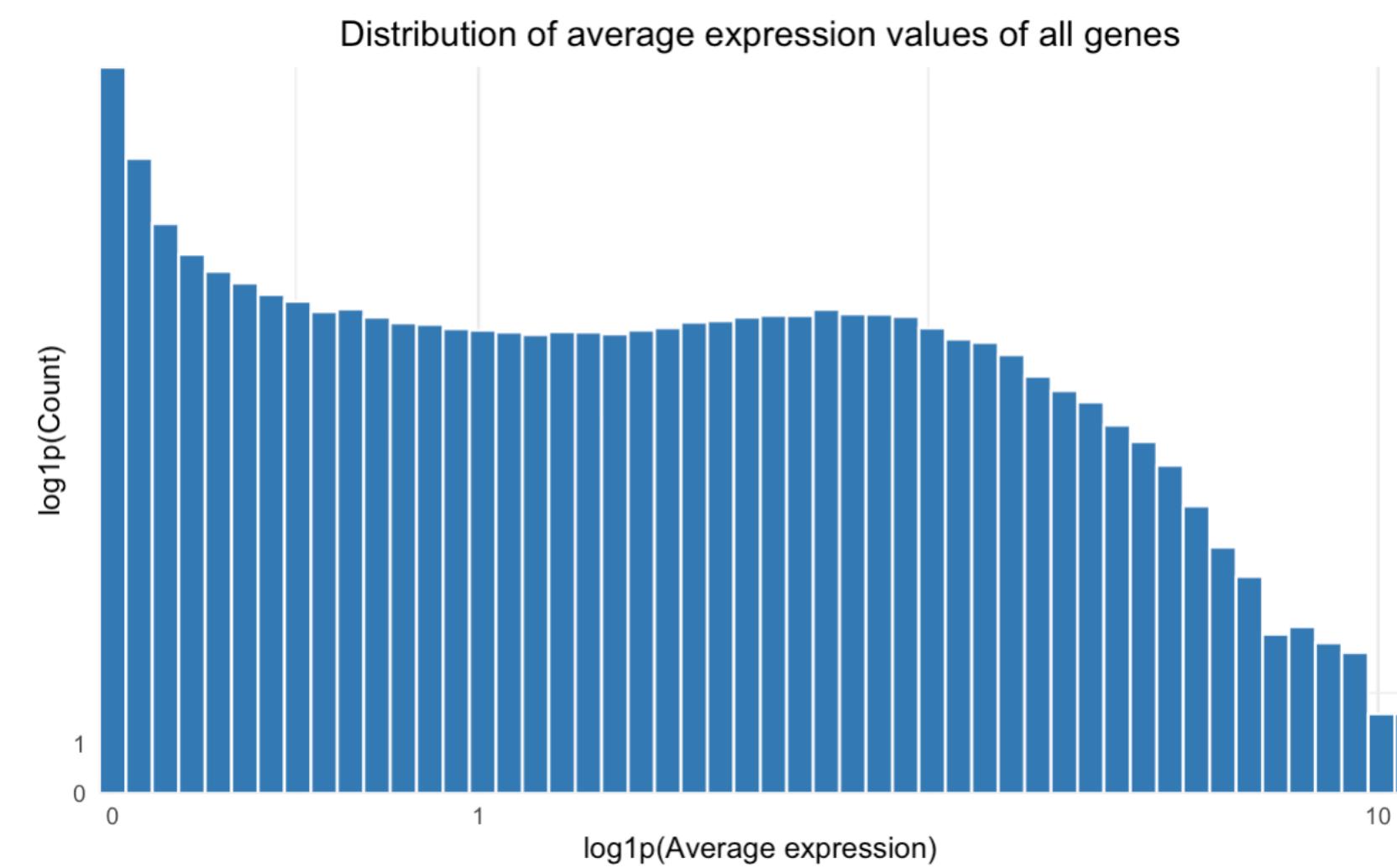
Number of input reads | 21176959
Average input read length | 245
UNIQUE READS:
    Uniquely mapped reads number | 16885614
    Uniquely mapped reads % | 79.74%
    Average mapped length | 247.84
    Number of splices: Total | 9658959
    Number of splices: Annotated (sjdb) | 9407590
    Number of splices: GT/AG | 9485975
    Number of splices: GC/AG | 73867
    Number of splices: AT/AC | 11223
    Number of splices: Non-canonical | 87894
    Mismatch rate per base, % | 0.44%
    Deletion rate per base | 0.02%
    Deletion average length | 1.87
    Insertion rate per base | 0.03%
    Insertion average length | 2.28
    MULTI-MAPPING READS:
        Number of reads mapped to multiple loci | 1694449
        % of reads mapped to multiple loci | 8.00%
        Number of reads mapped to too many loci | 62165
        % of reads mapped to too many loci | 0.29%
    UNMAPPED READS:
        Number of reads unmapped: too many mismatches | 0
        % of reads unmapped: too many mismatches | 0.00%
        Number of reads unmapped: too short | 2469842
        % of reads unmapped: too short | 11.66%
        Number of reads unmapped: other | 64889
        % of reads unmapped: other | 0.31%
    CHIMERIC READS:
        Number of chimeric reads | 0
        % of chimeric reads | 0.00%
```

Uniquely mapped reads % | 79.74%

% of reads mapped to multiple loci | 8.00%

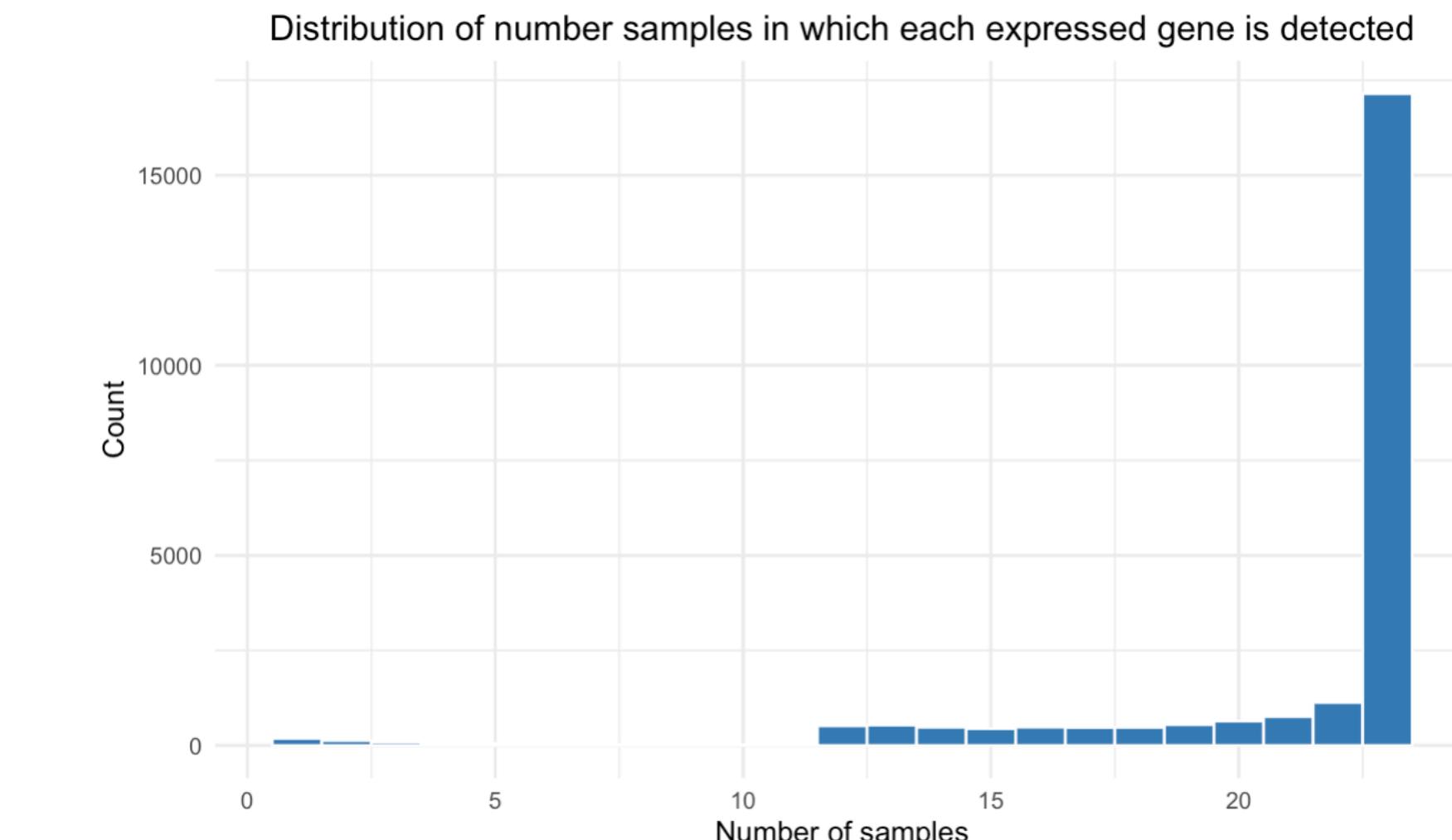
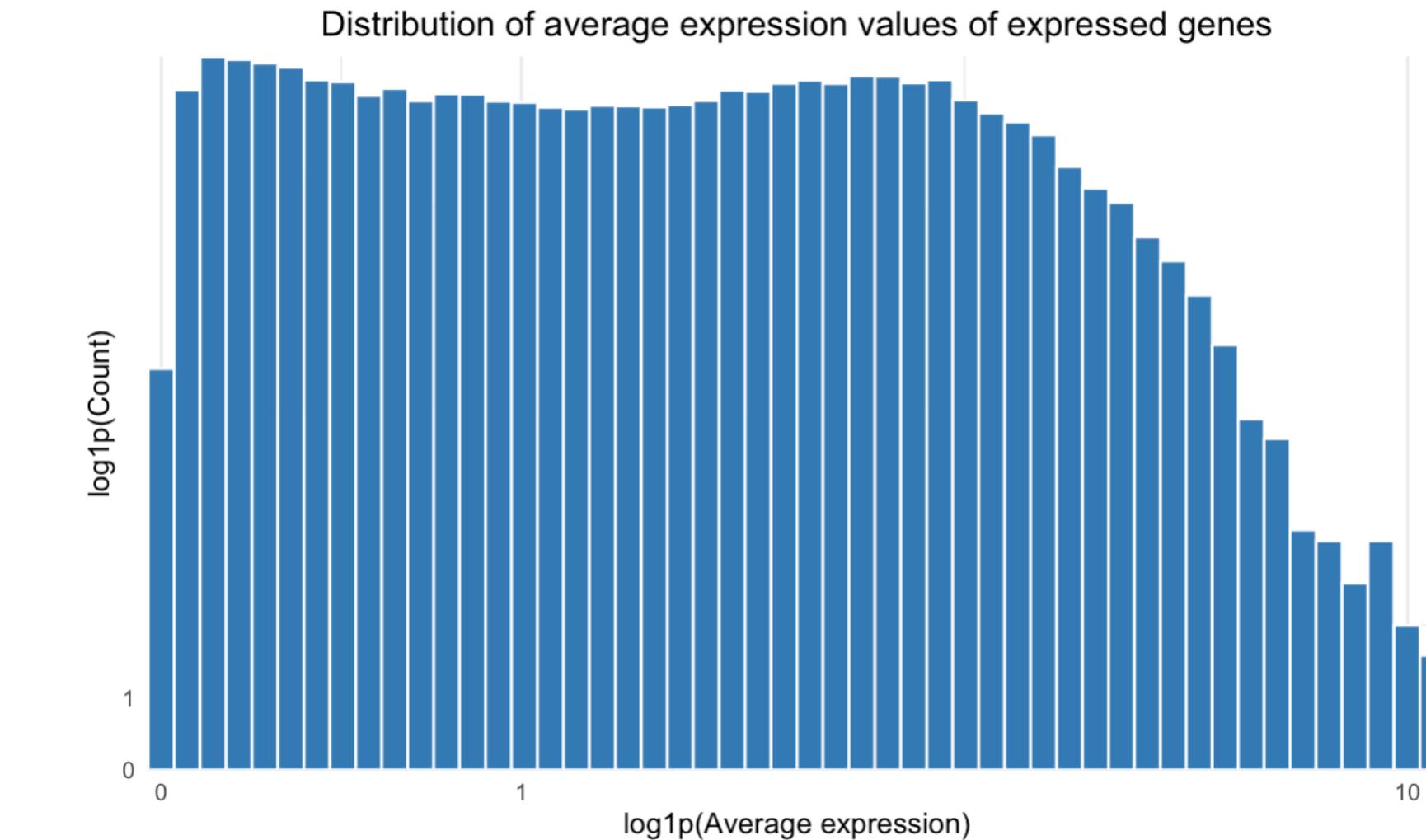
Exploratory analysis

Gene Expression



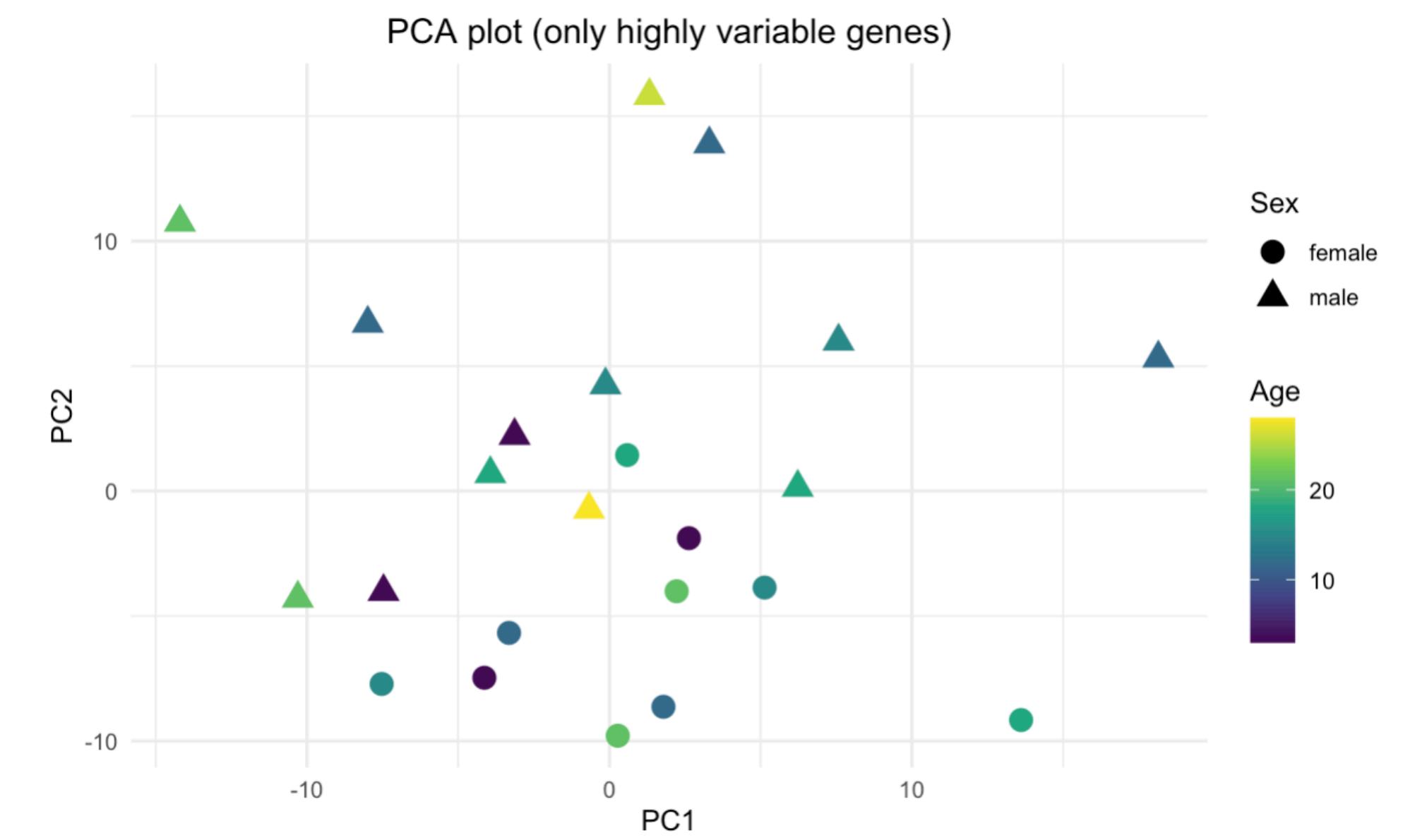
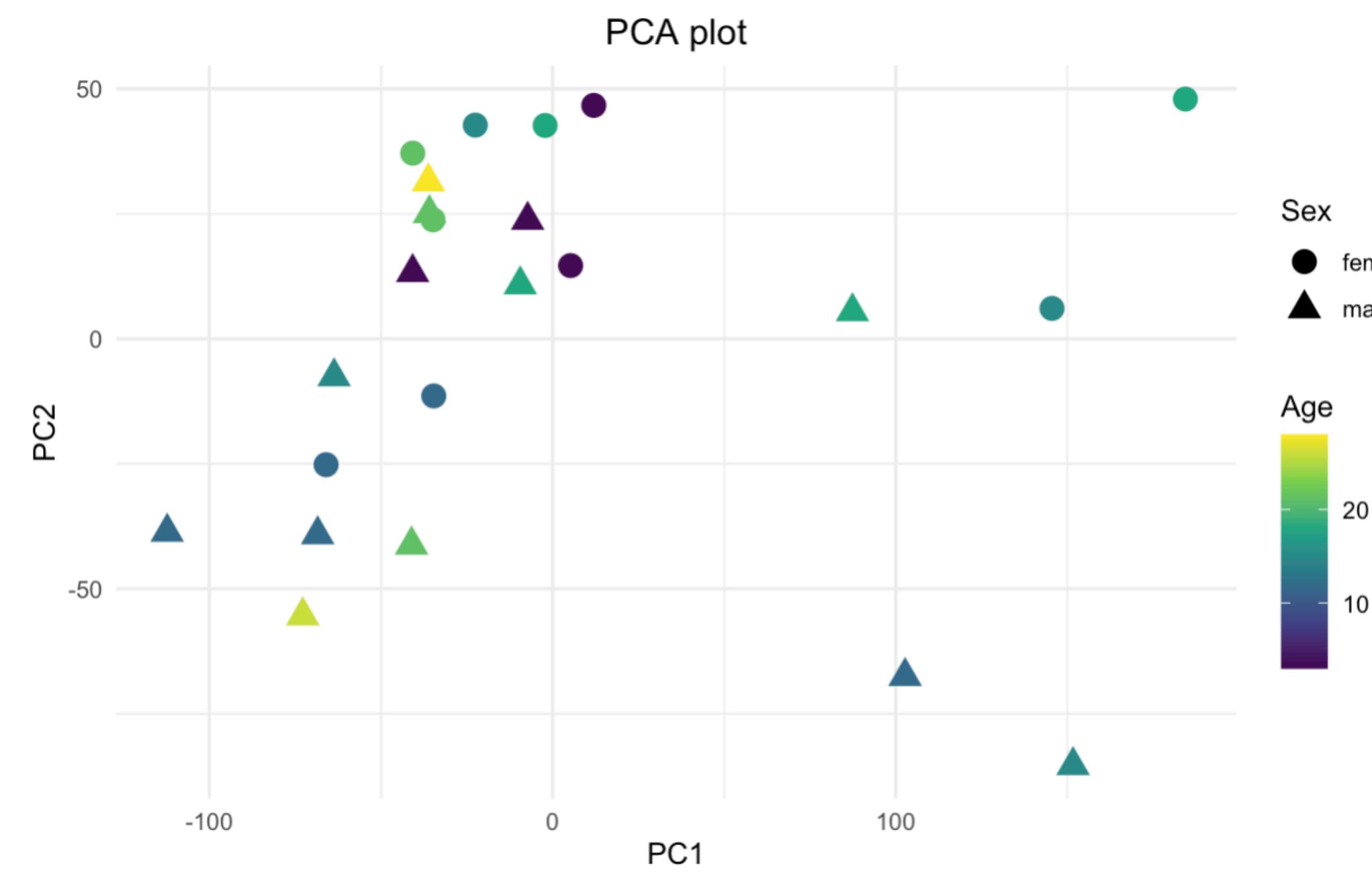
After filtering

- Filtering criteria:**
- Average TPM ≥ 1
 - Or genes detected in at least 1/2 of the samples.



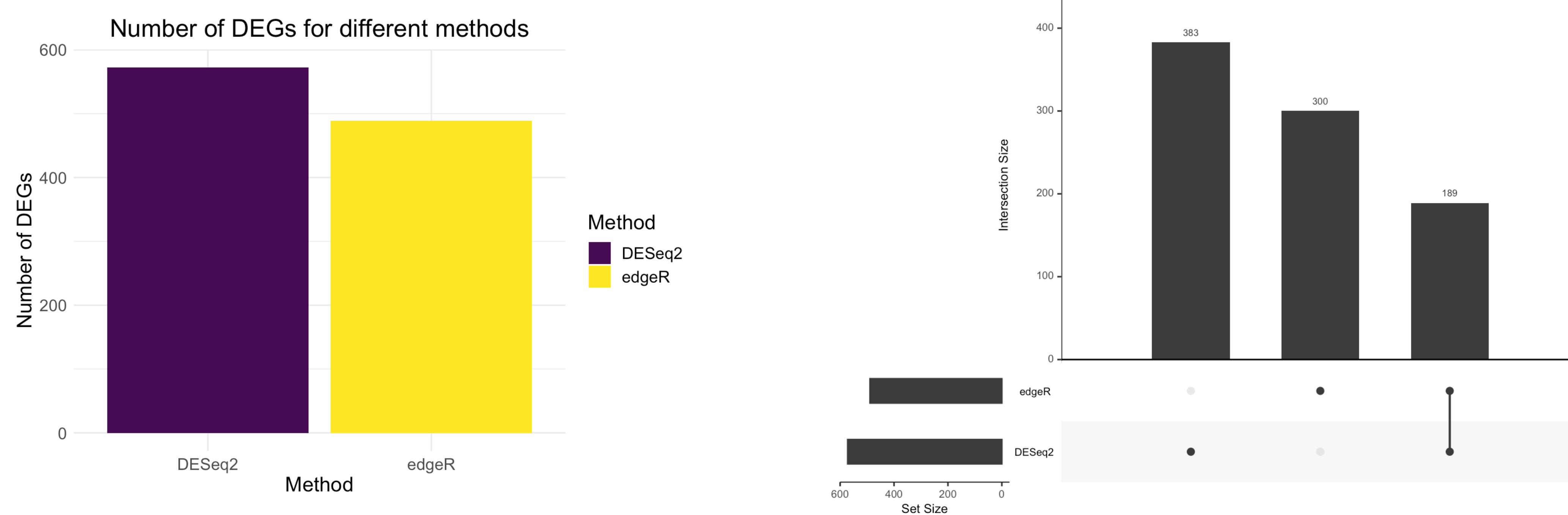
Exploratory analysis

Clustering and Dimensionality Reduction



DE Analysis

Detection of DEGs over the course of aging



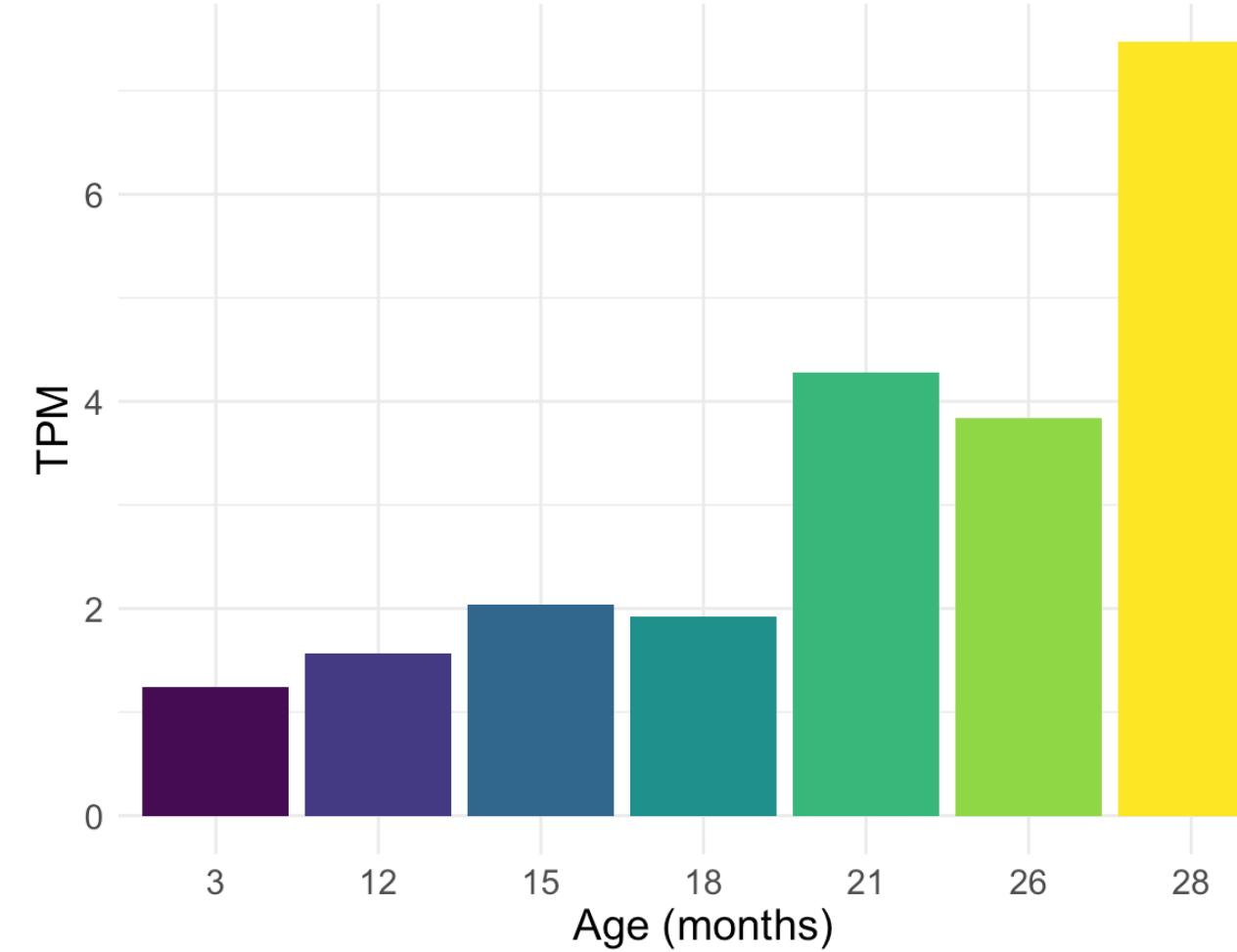
Genes are considered as DE if the adjusted p-value of the LRT is below 0.1

Many genes detected by both methods, but also many detected uniquely by one only!

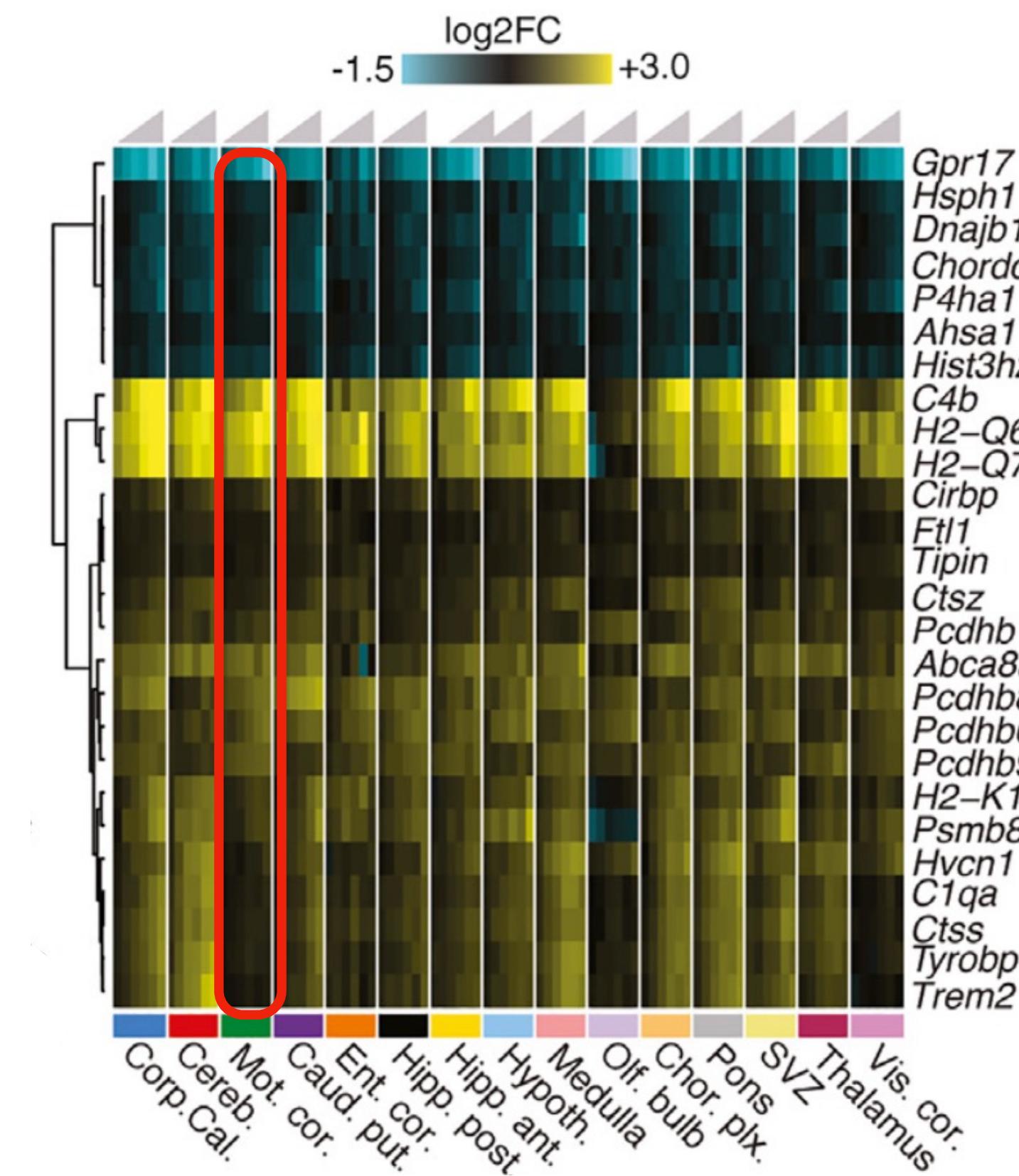
DE Analysis

Important genes are detected by both methods

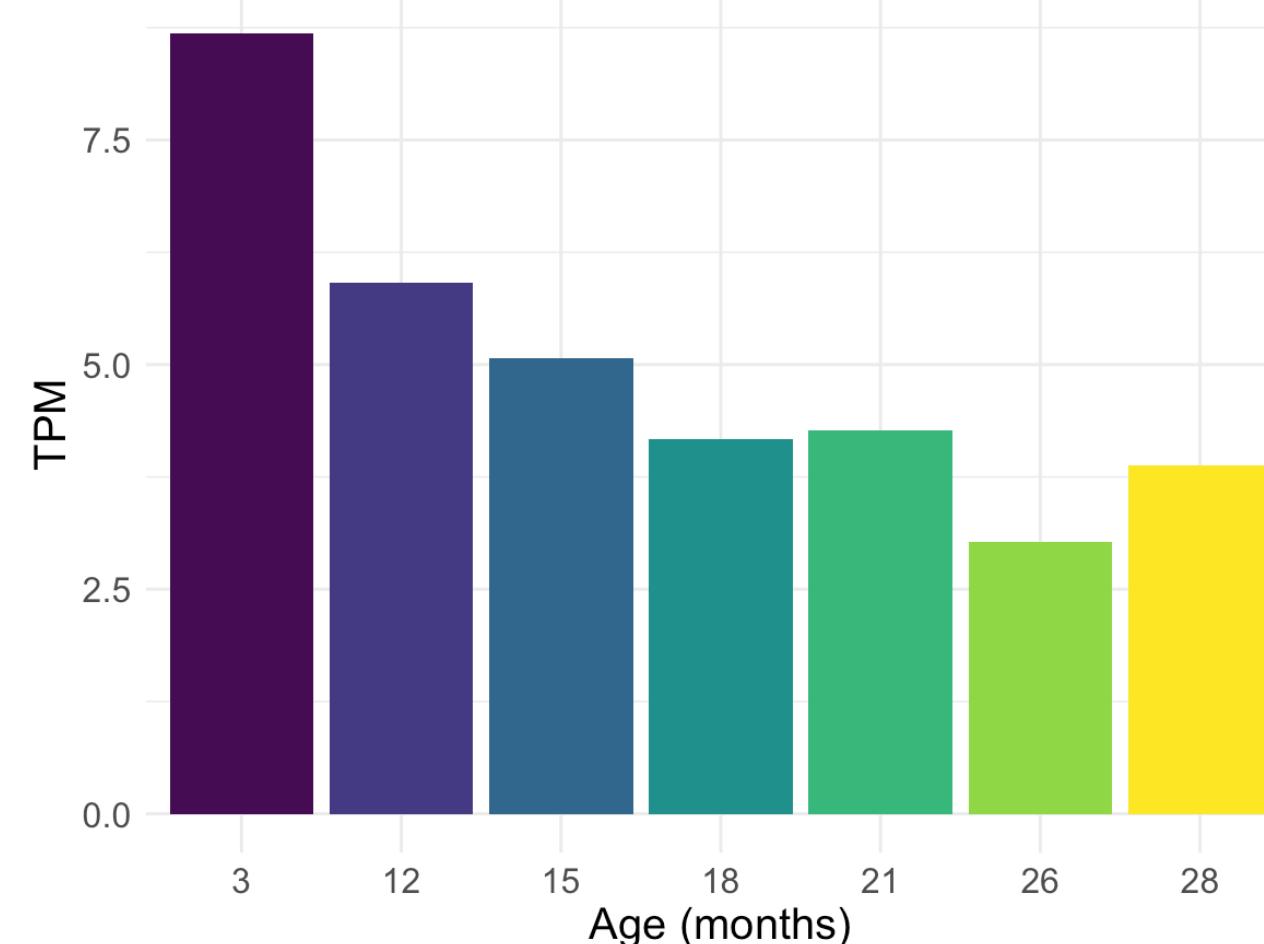
Expression of C4b over time



Age
3
12
15
18
21
26
28

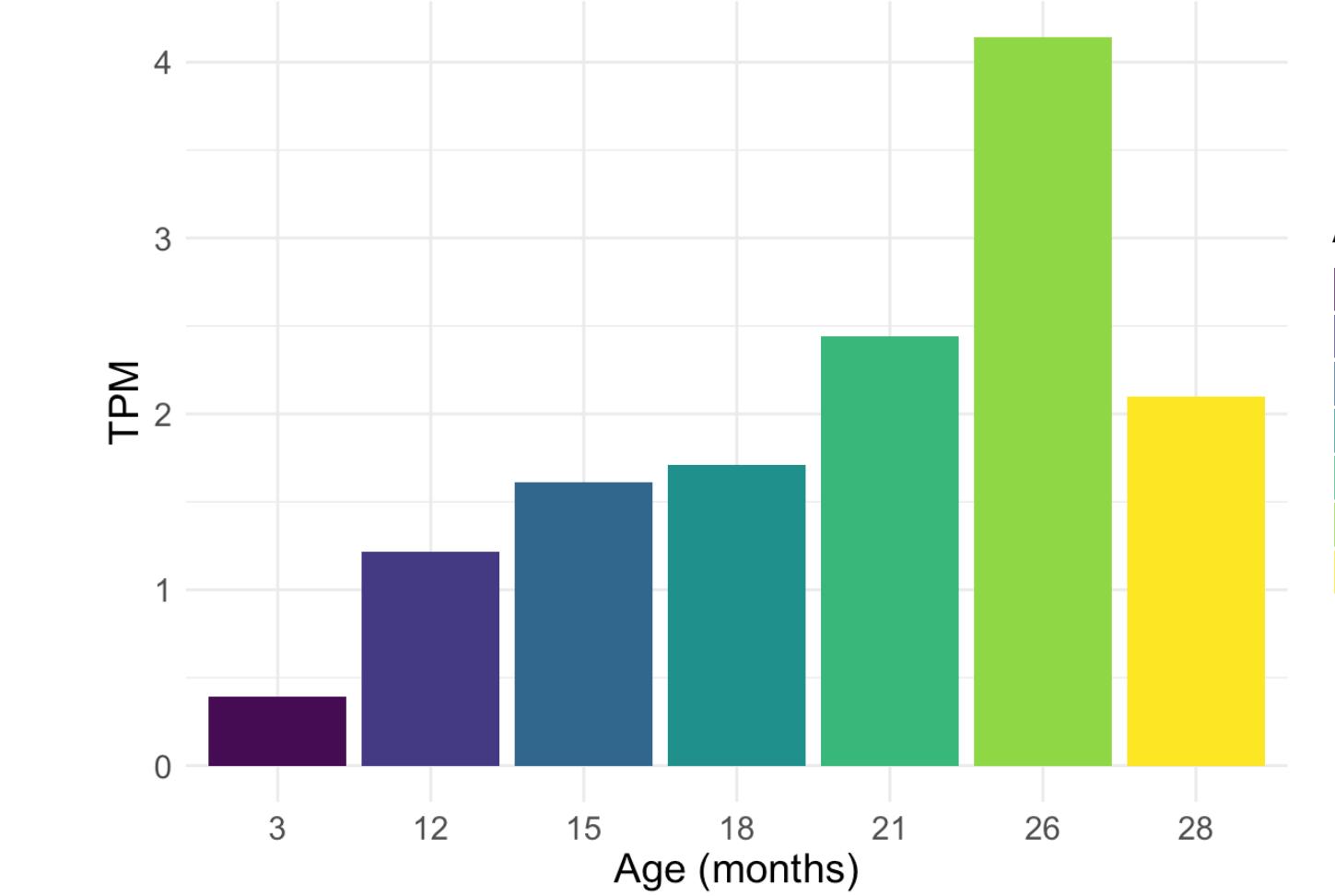


Expression of Gpr17 over time



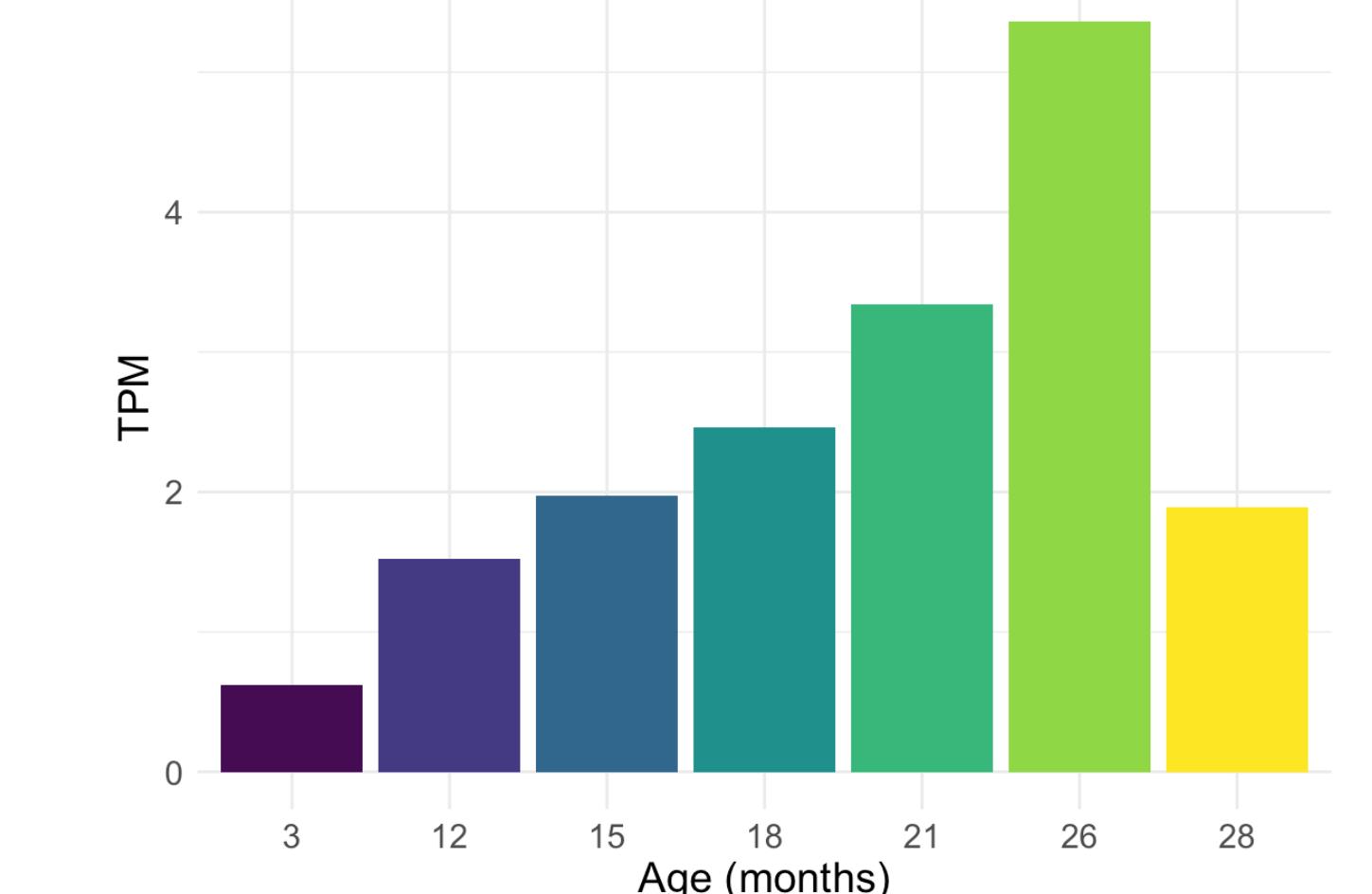
Age
3
12
15
18
21
26
28

Expression of H2-Q6 over time



Age
3
12
15
18
21
26
28

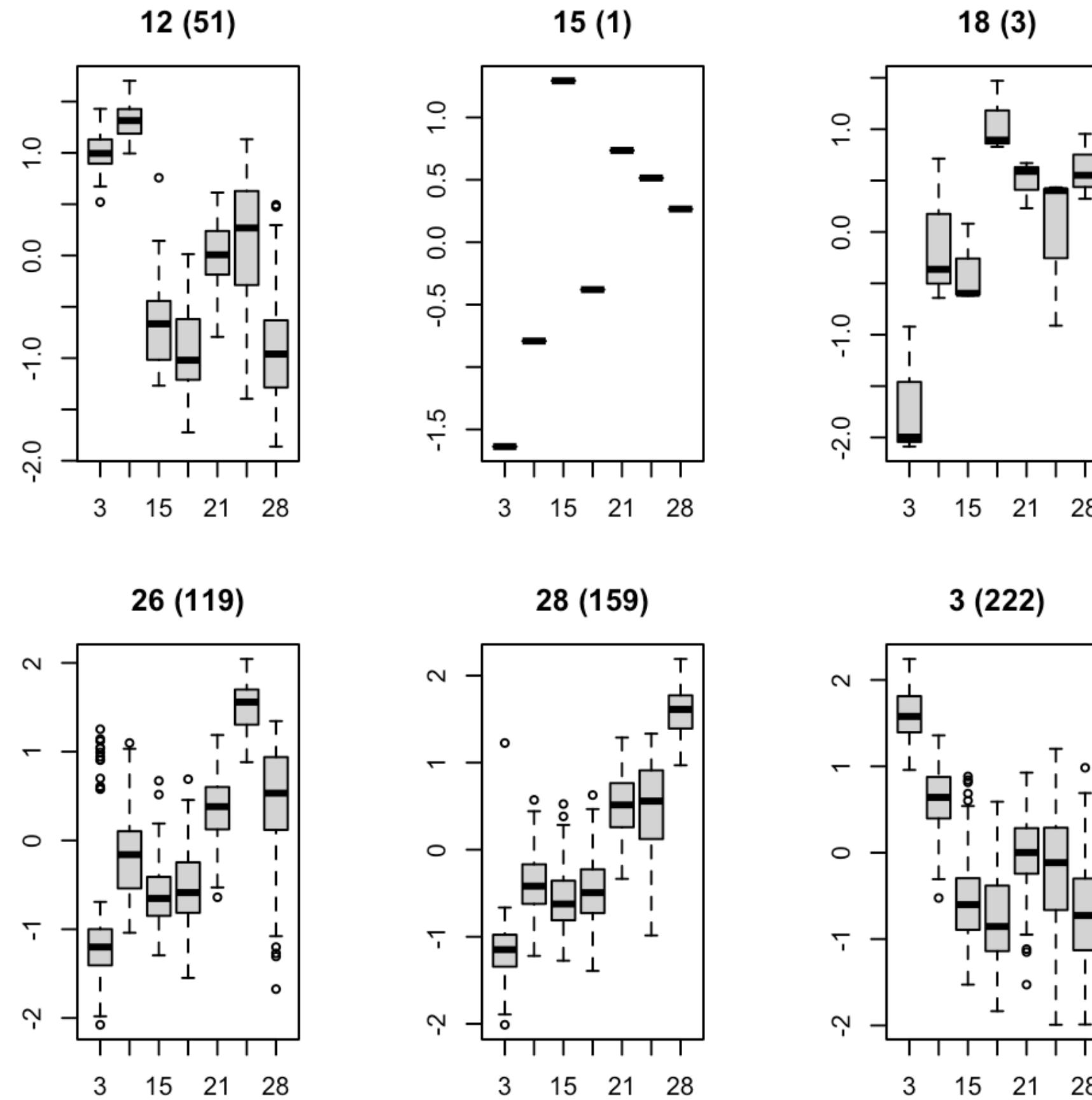
Expression of H2-Q7 over time



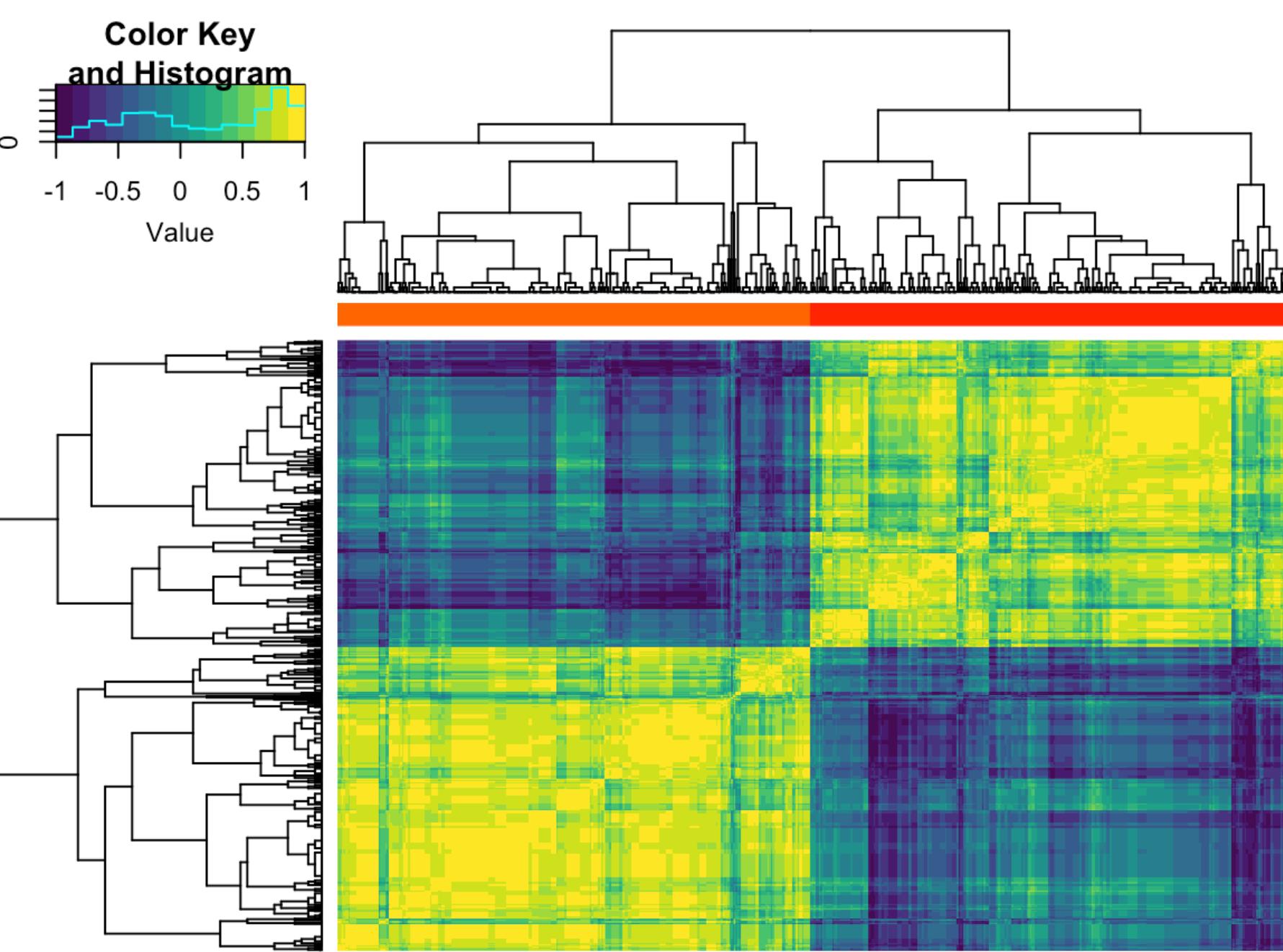
Age
3
12
15
18
21
26
28

Grouping DEGs

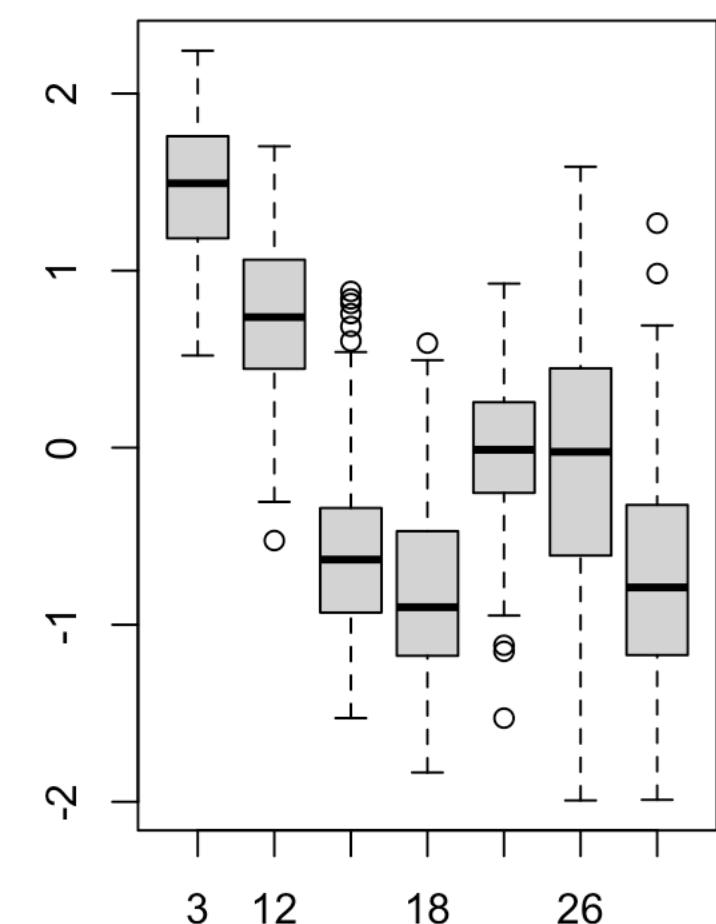
DESeq2 - continuous time



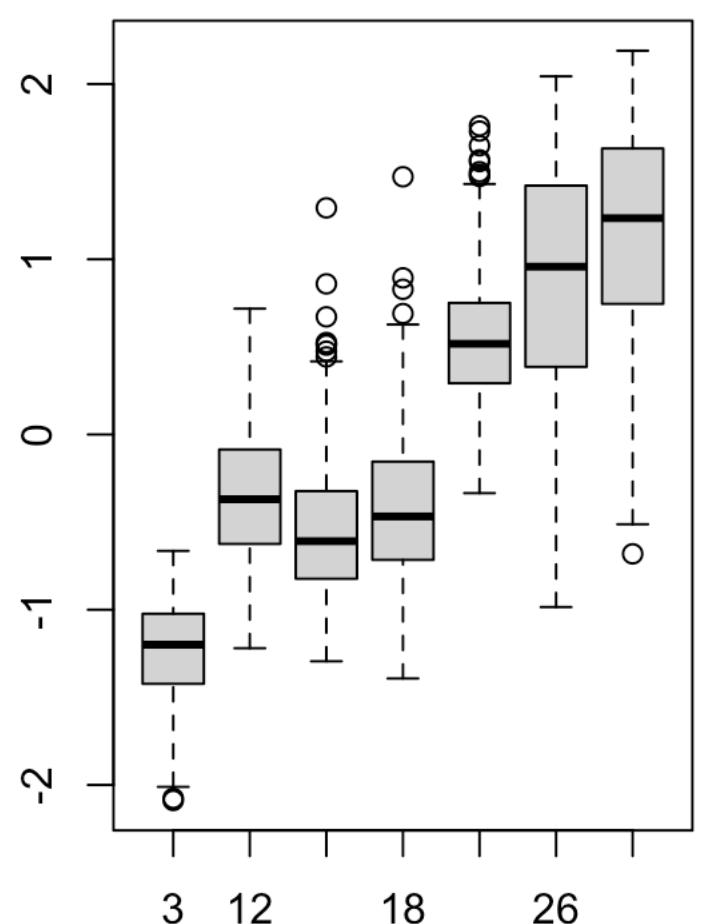
3 (222)



1 (287)



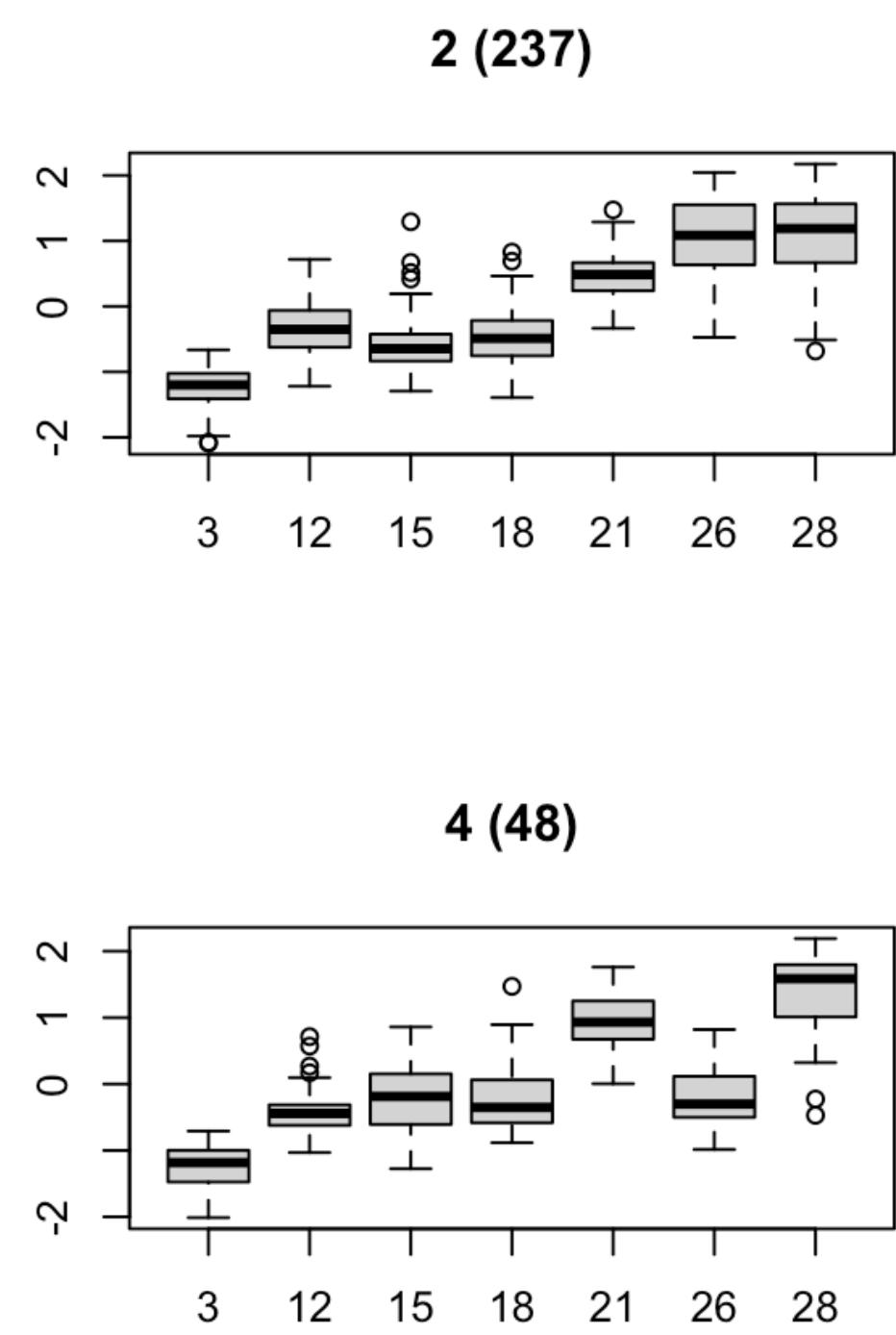
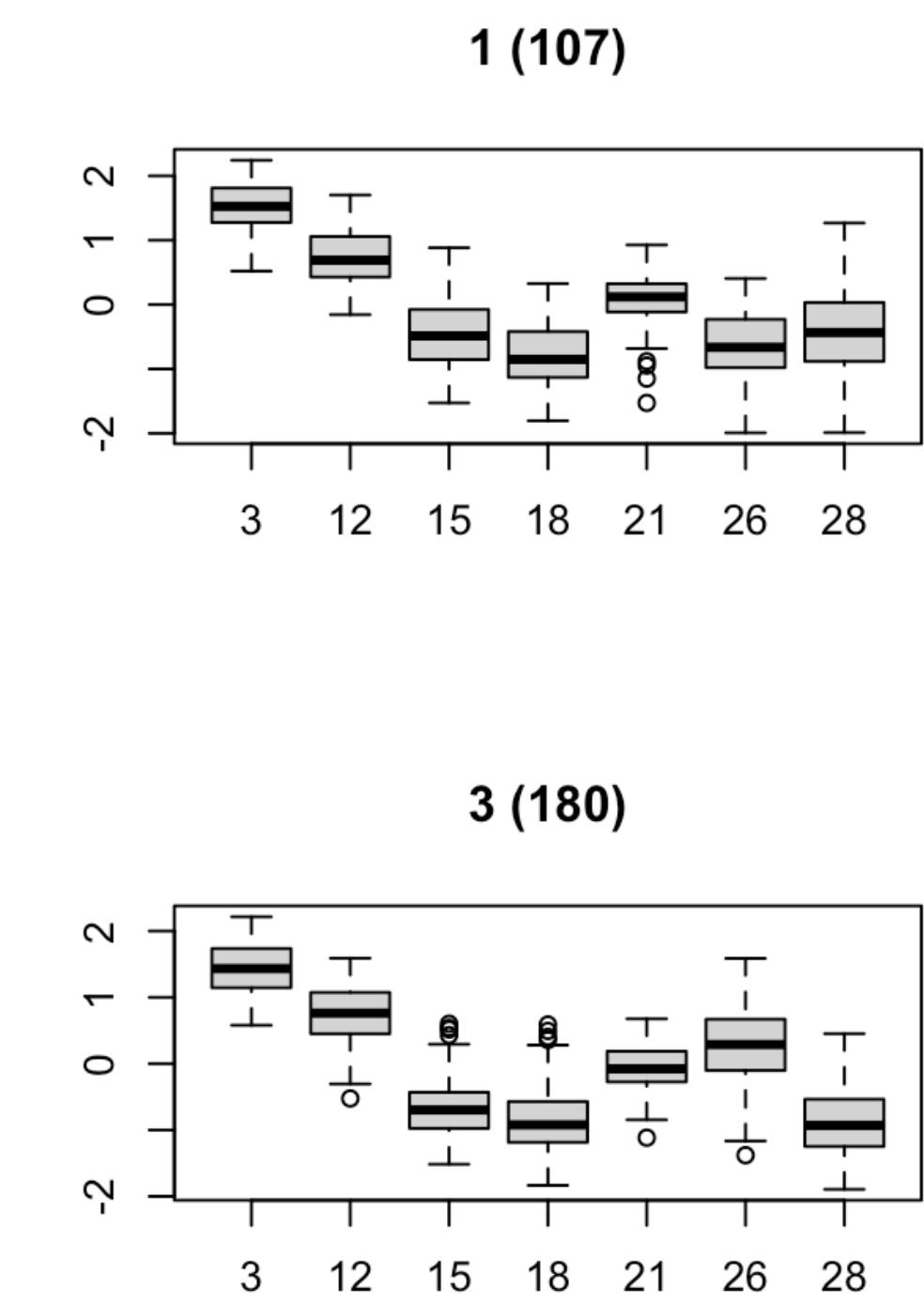
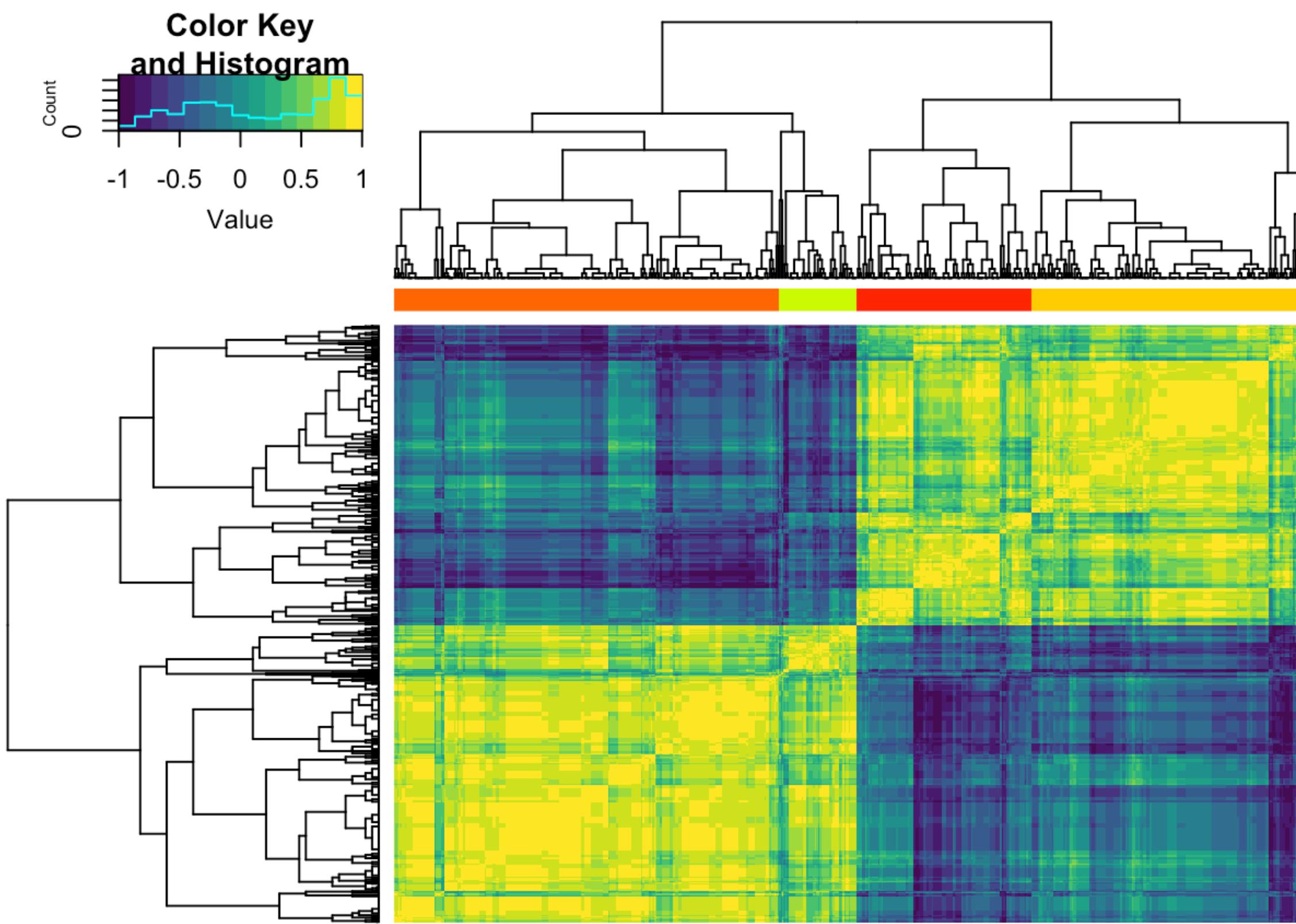
2 (285)



Most DEGs have their highest average expression either at low or high age!

Grouping DEGs

DESeq2 - clusters of DEGs



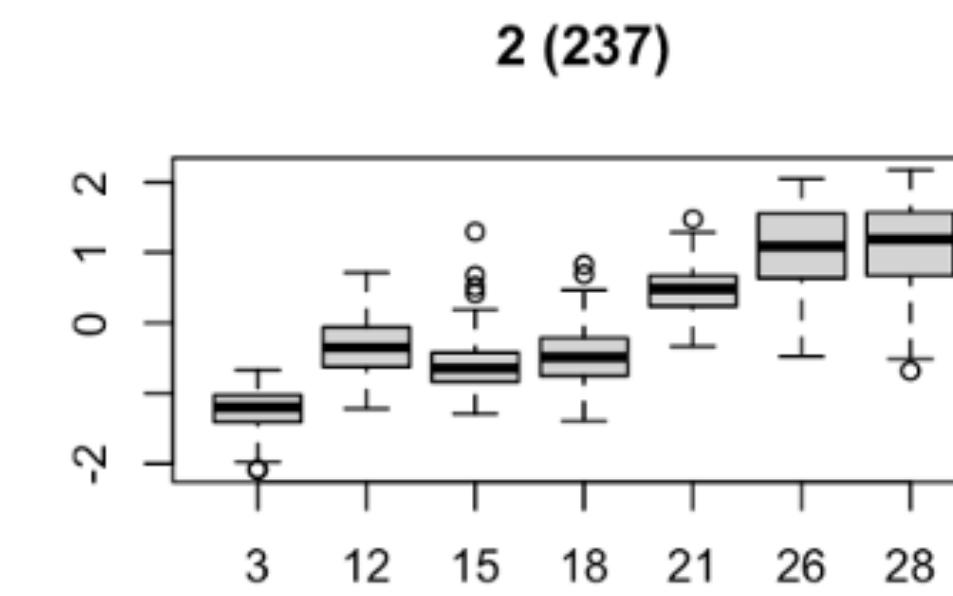
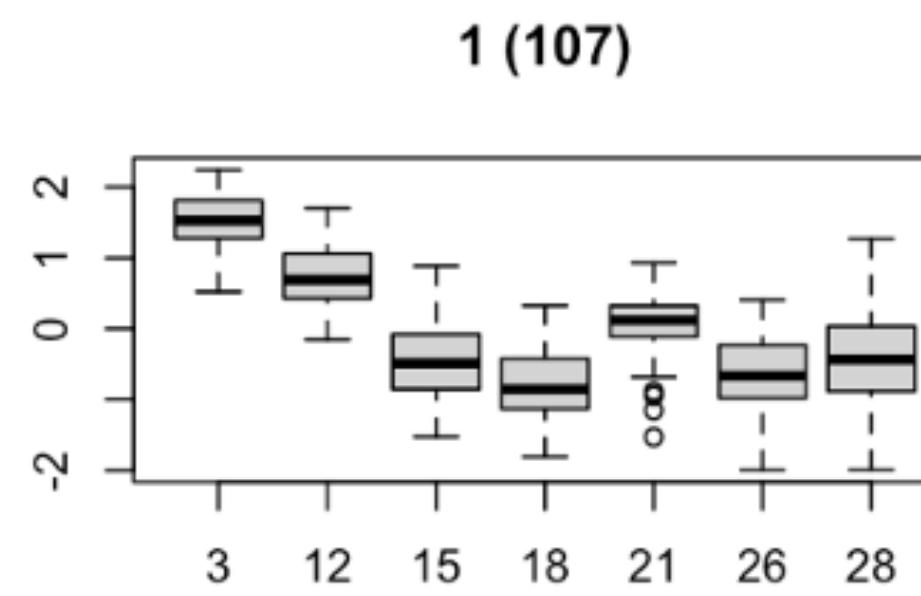
Gene Enrichment

Clusters - Pathways and processes



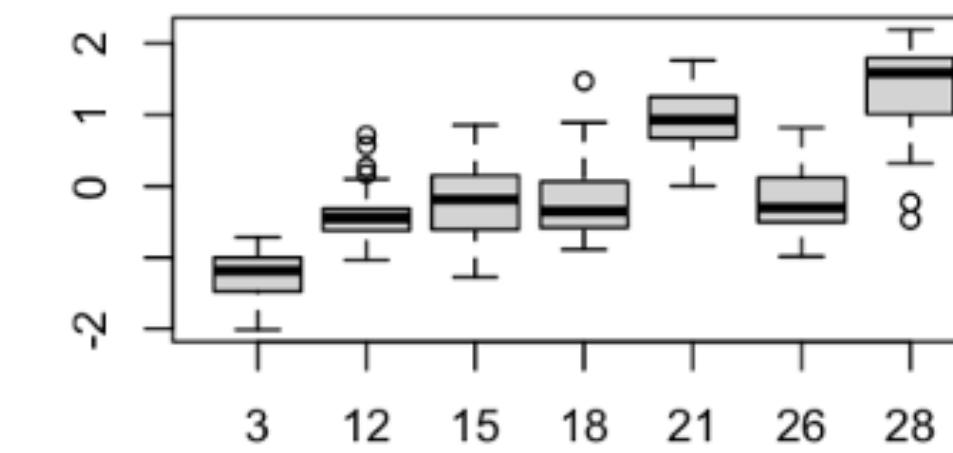
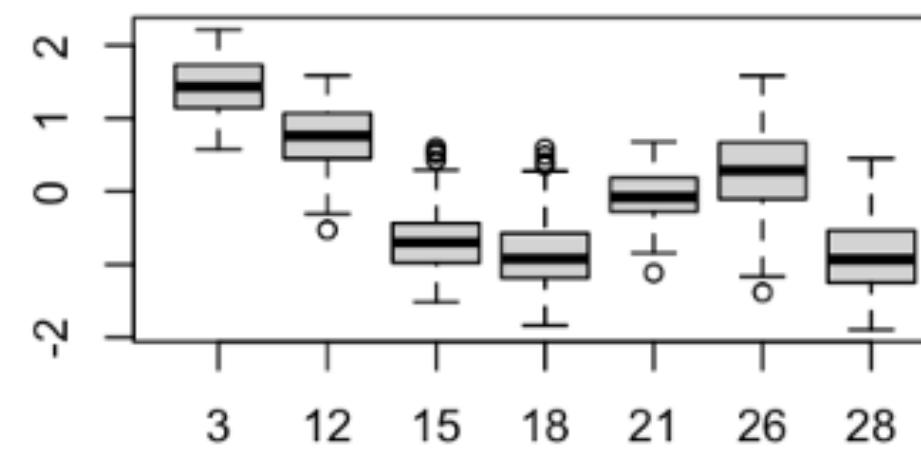
GORILLA

Differentiation and development of neuronal cells



Pathways of the immune system

Cell morphogenesis



Regulation of myelination

Summary

Age-dependent transcriptomic changes in the Motor Cortex

- Quantification of bulk RNA-seq revealed **24.357 expressed genes**.
- DE analysis with DESeq2 showed **572 aging-related genes** in the Motor Cortex.
- As shown by **GE**, these genes are involved in:
 - Differentiation and development of neural cells
 - Pathways of the immune system
 - Cell morphogenesis
 - Myelination

Thank you for your attention!

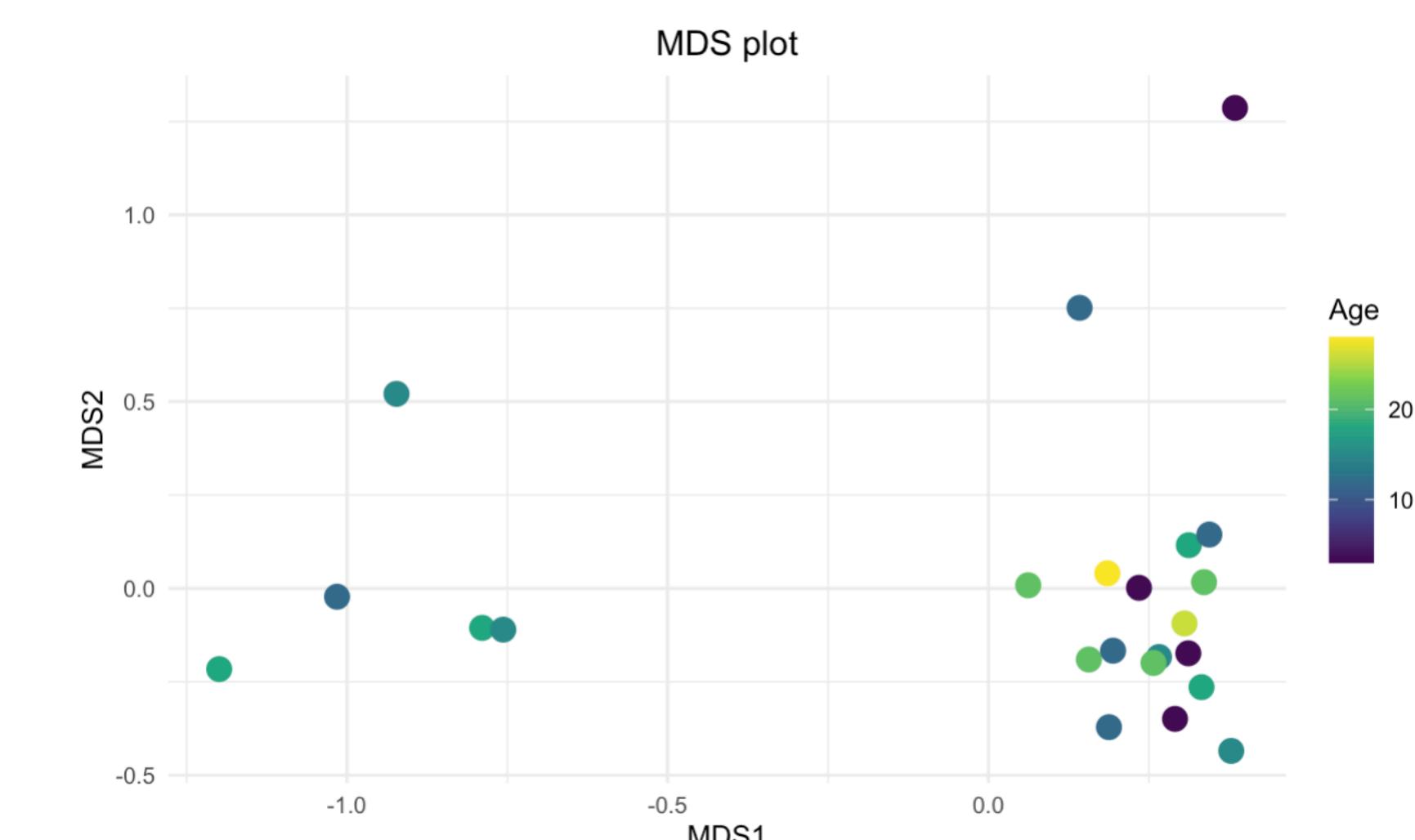
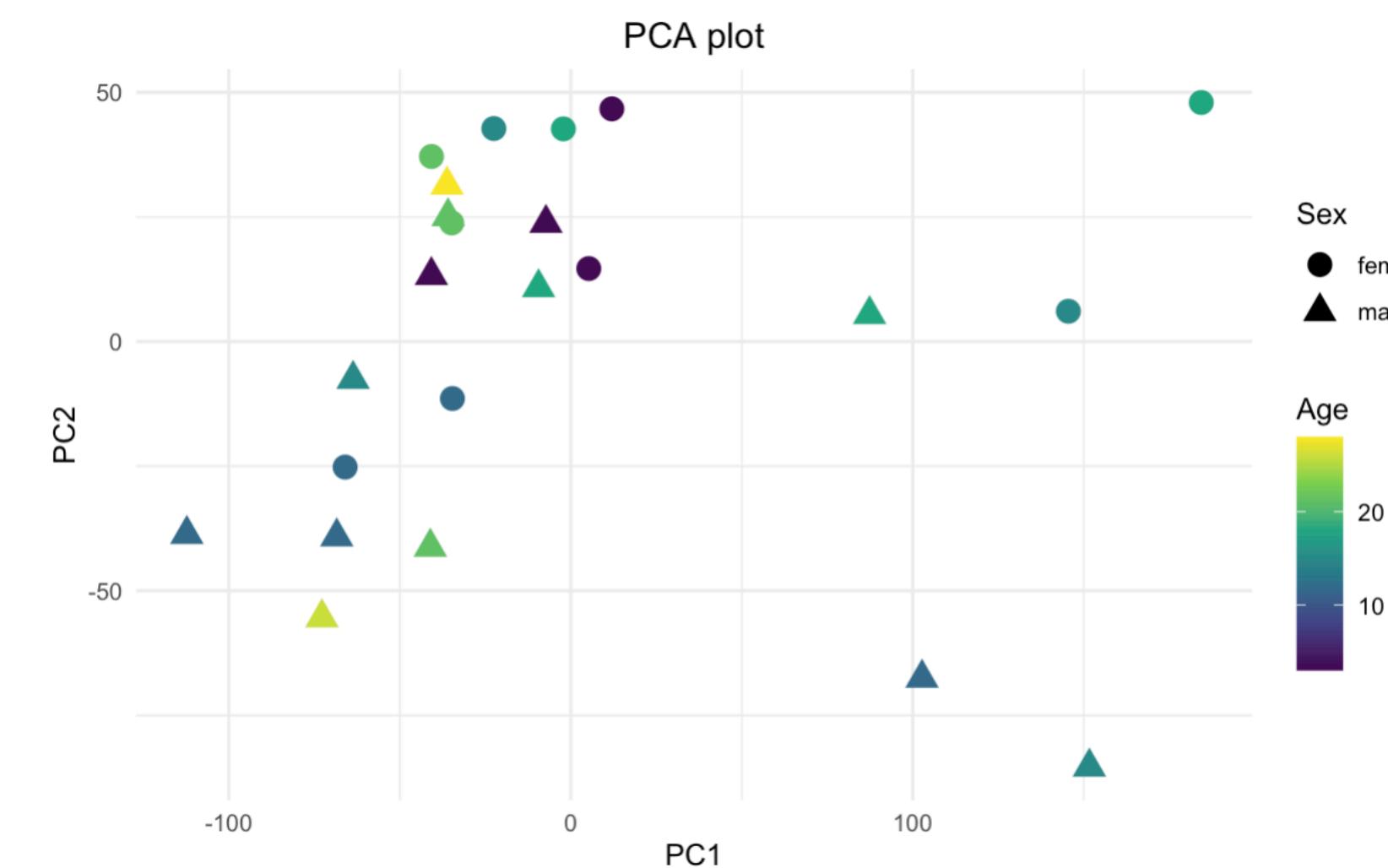
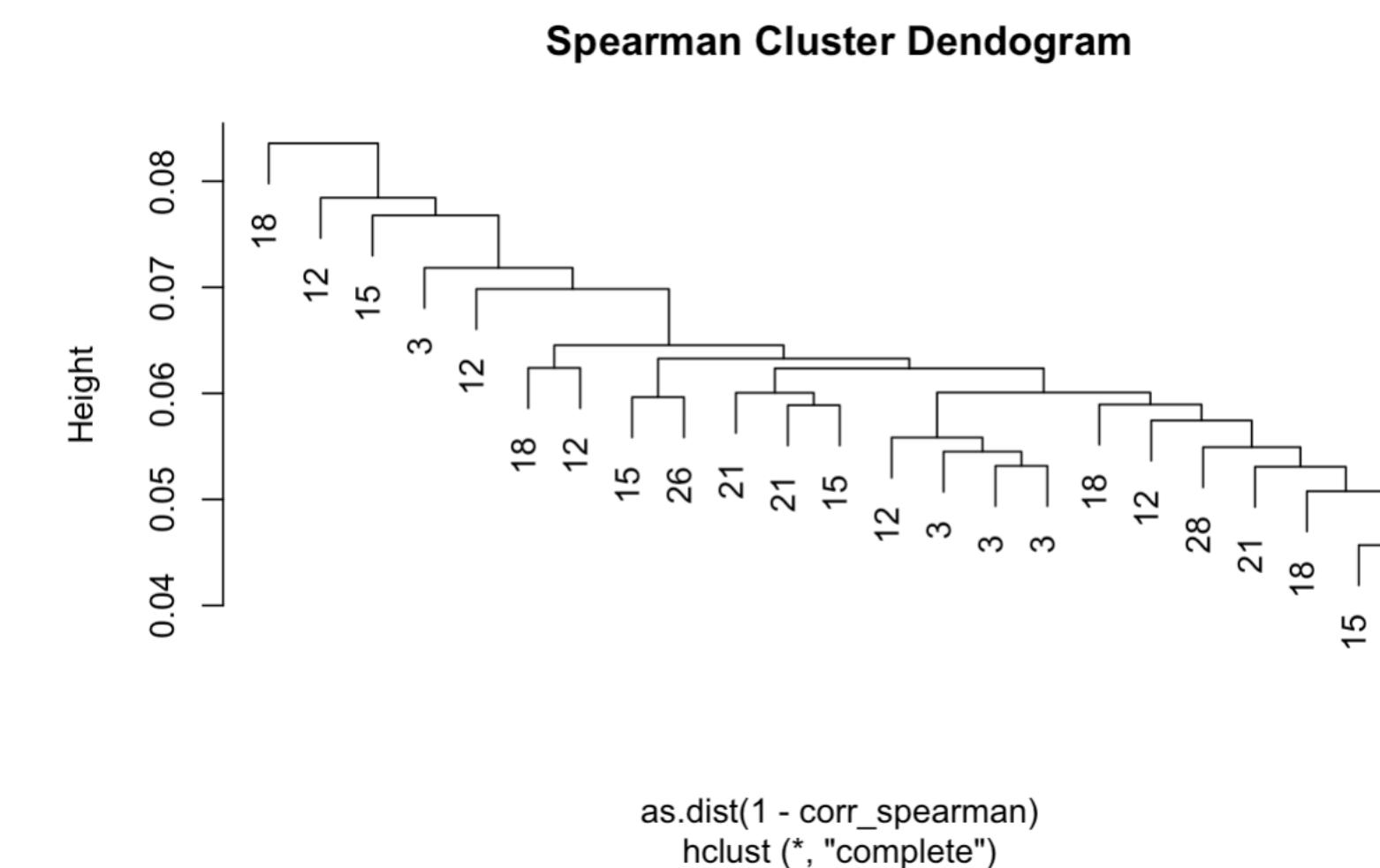
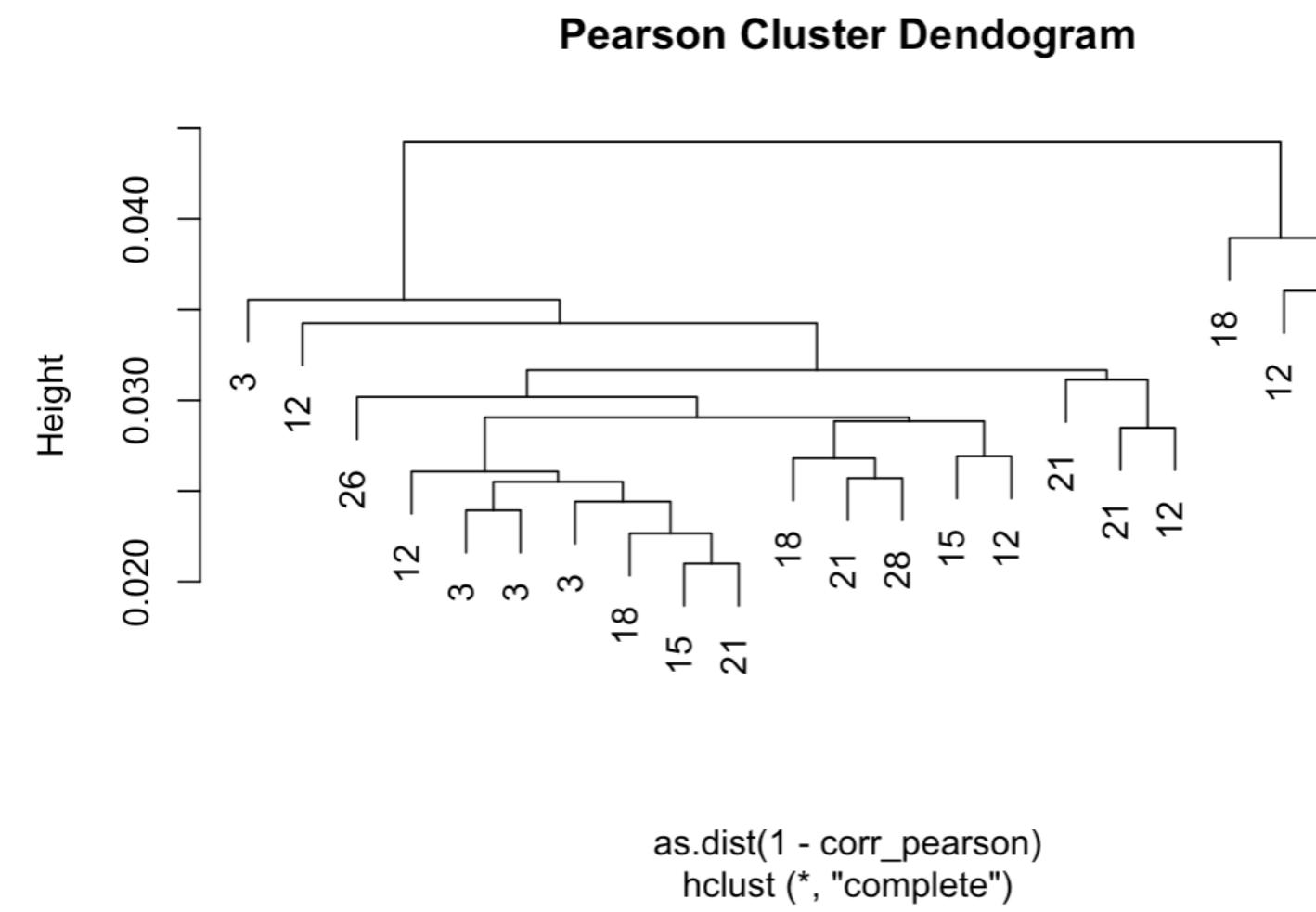


Sources

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- Woo, J. J., Pouget, J. G., Zai, C. C., & Kennedy, J. L. (2020). The complement system in schizophrenia: where are we now and what's next?. *Molecular psychiatry*, 25(1), 114–130. <https://doi.org/10.1038/s41380-019-0479-0>
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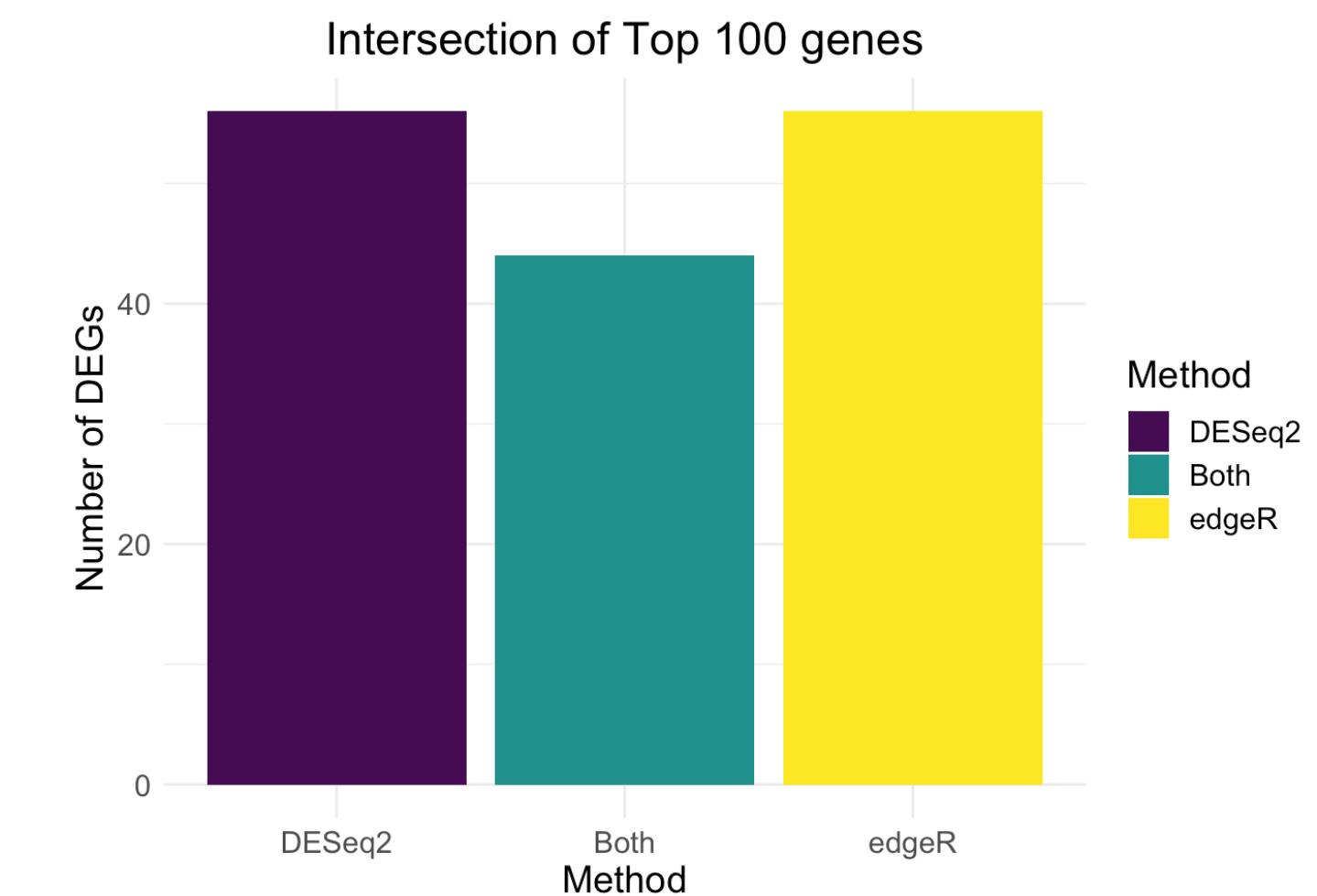
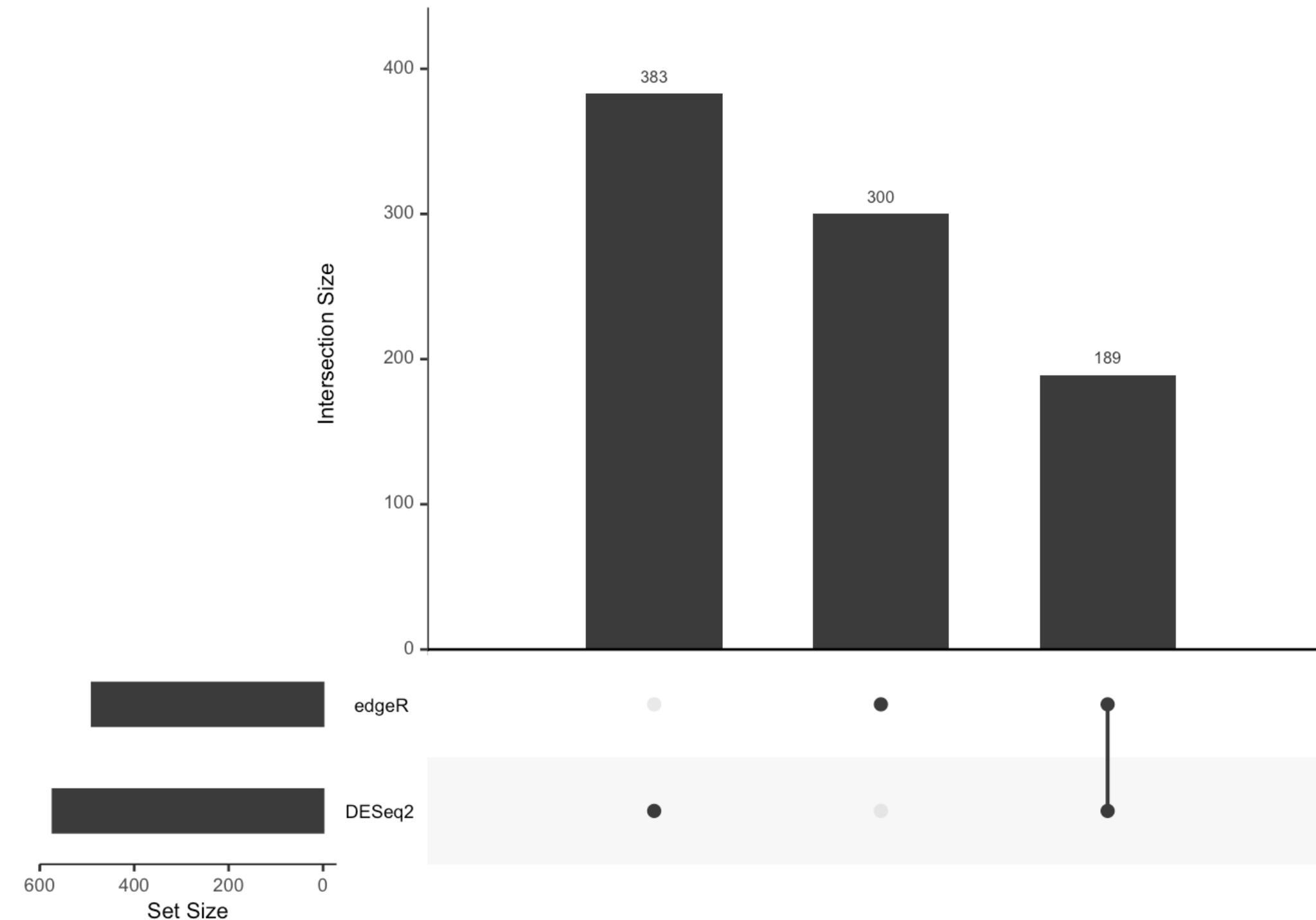
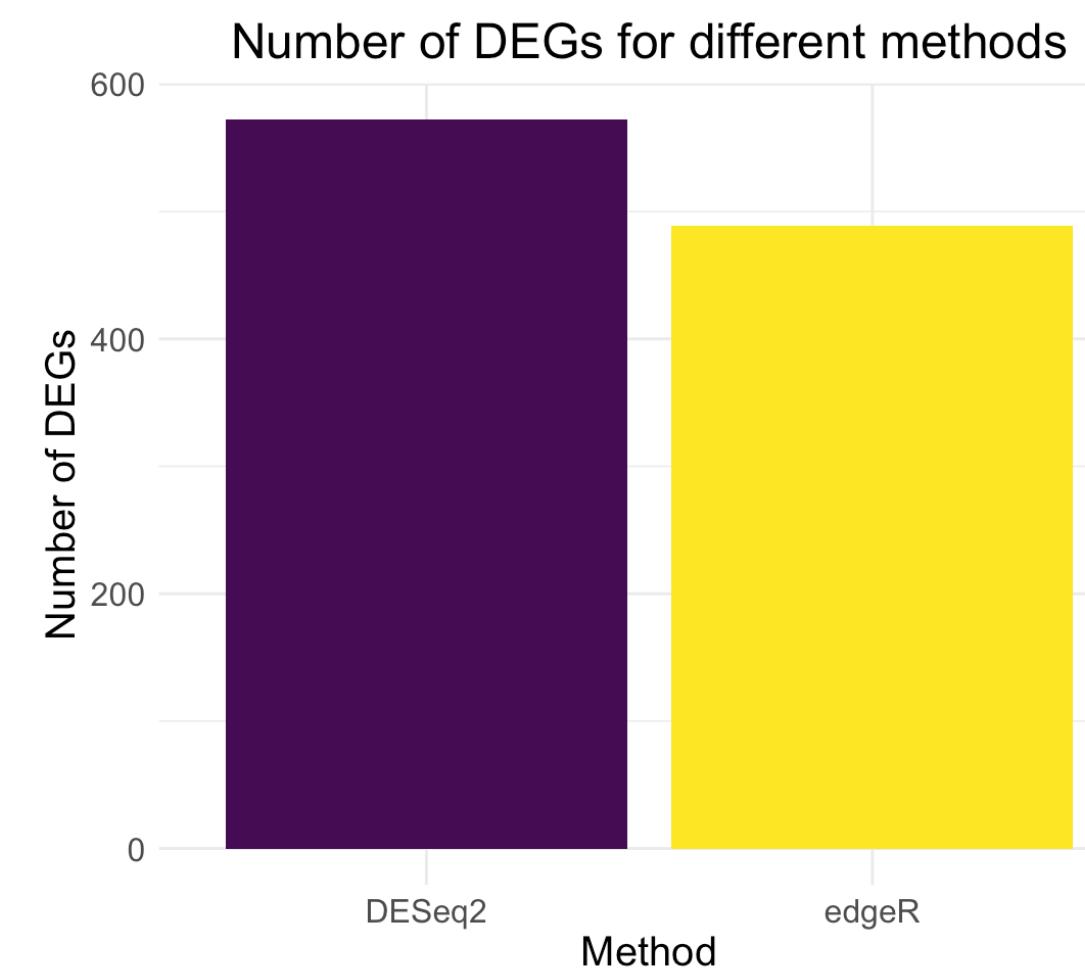
Exploratory analysis

Clustering and Dimensionality Reduction



DE Analysis

Detection of DE genes over the course of aging

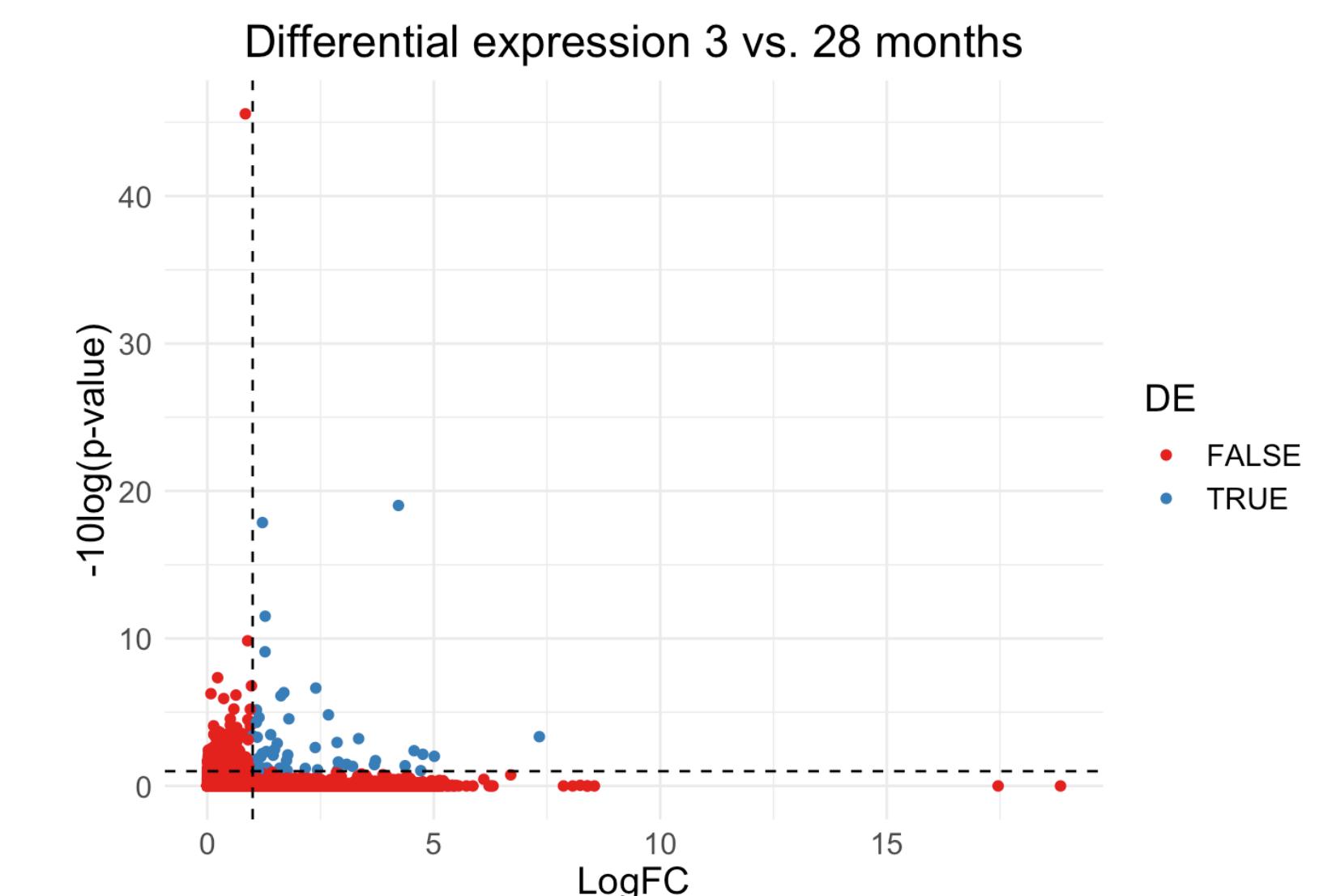
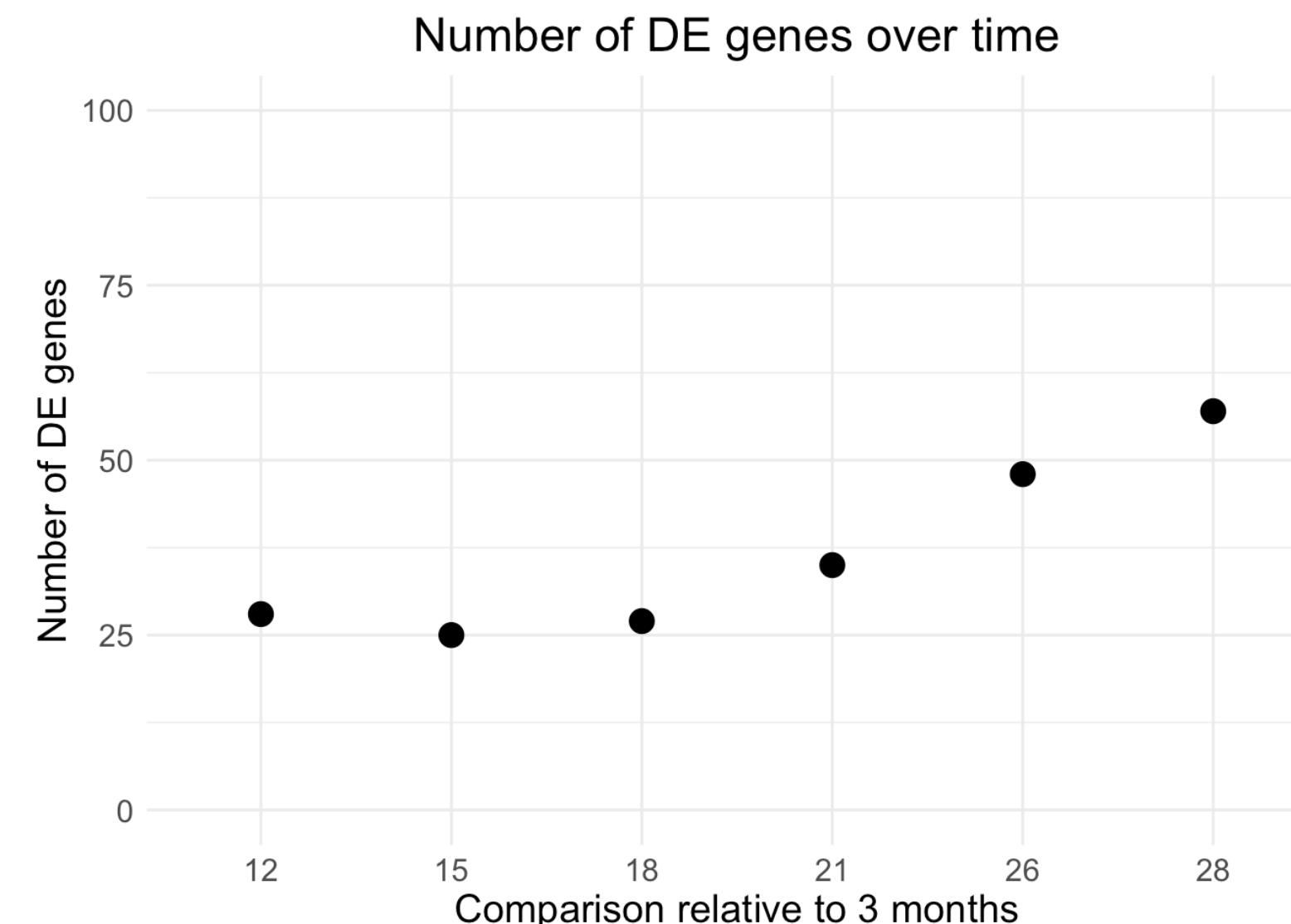
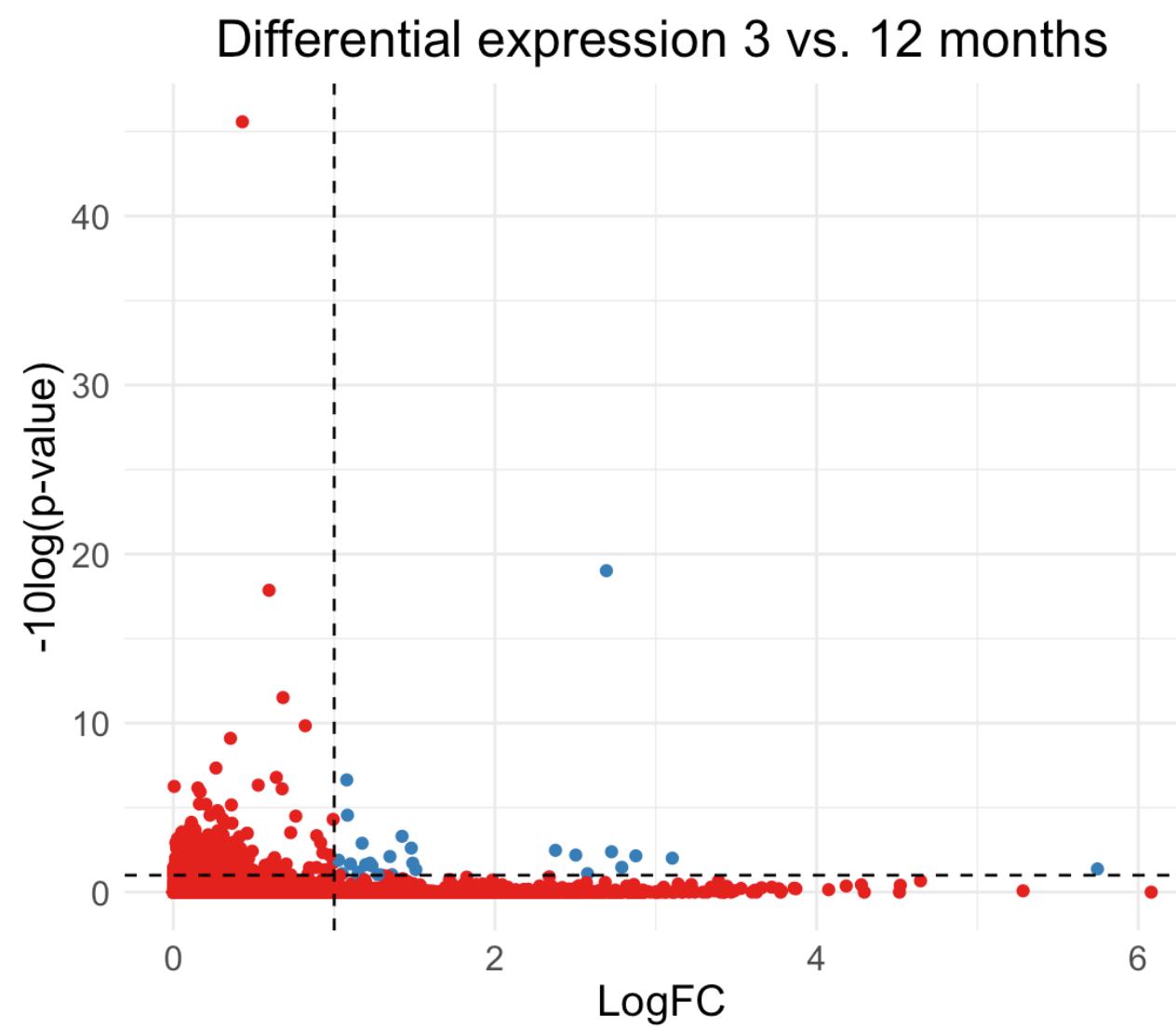


Genes are considered as DE if the adjusted p-value of the LRT is below 0.1

Many genes detected by both methods, but also many detected uniquely by one only!

DE Analysis

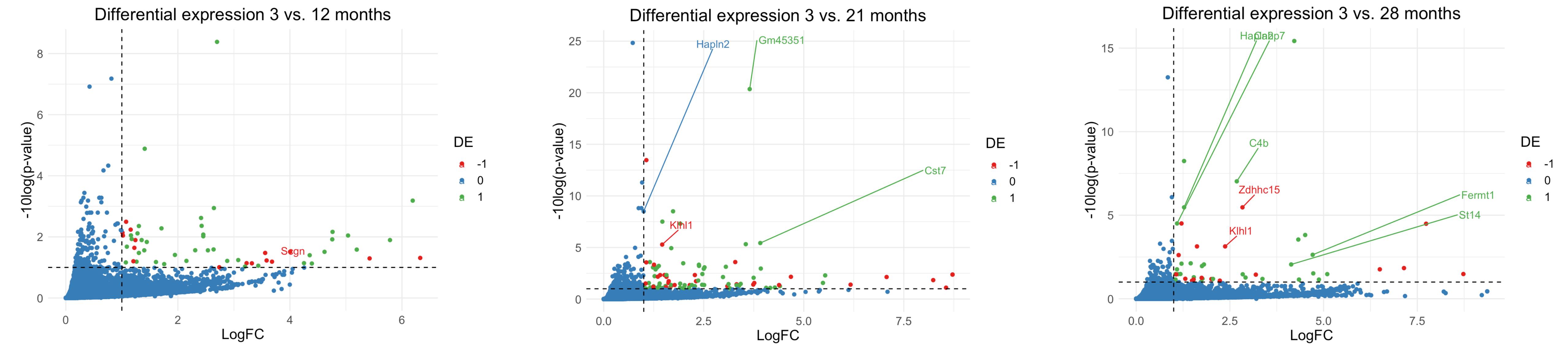
DESeq2 - discrete time points



Number of DEGs seems to increase over time

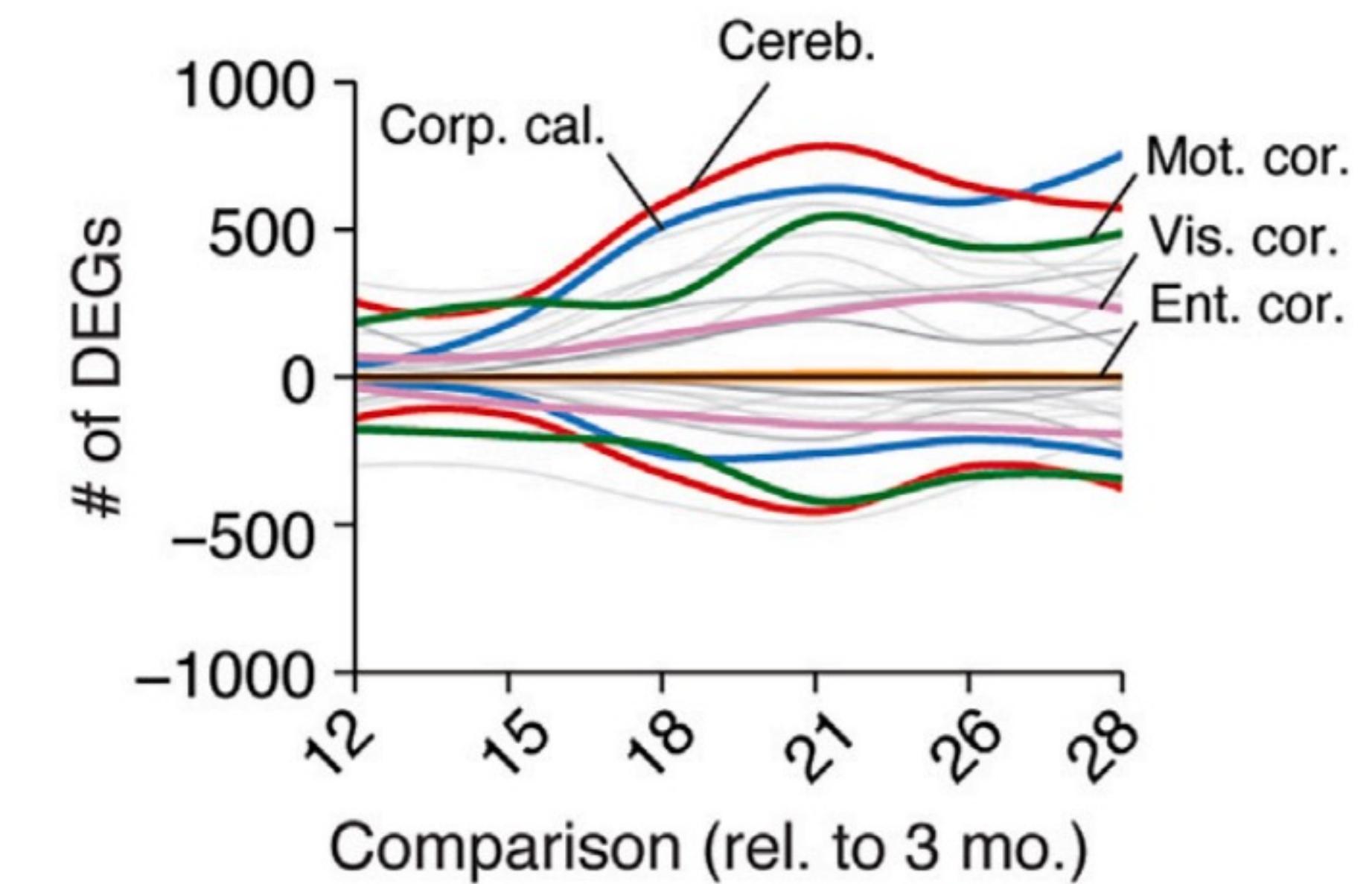
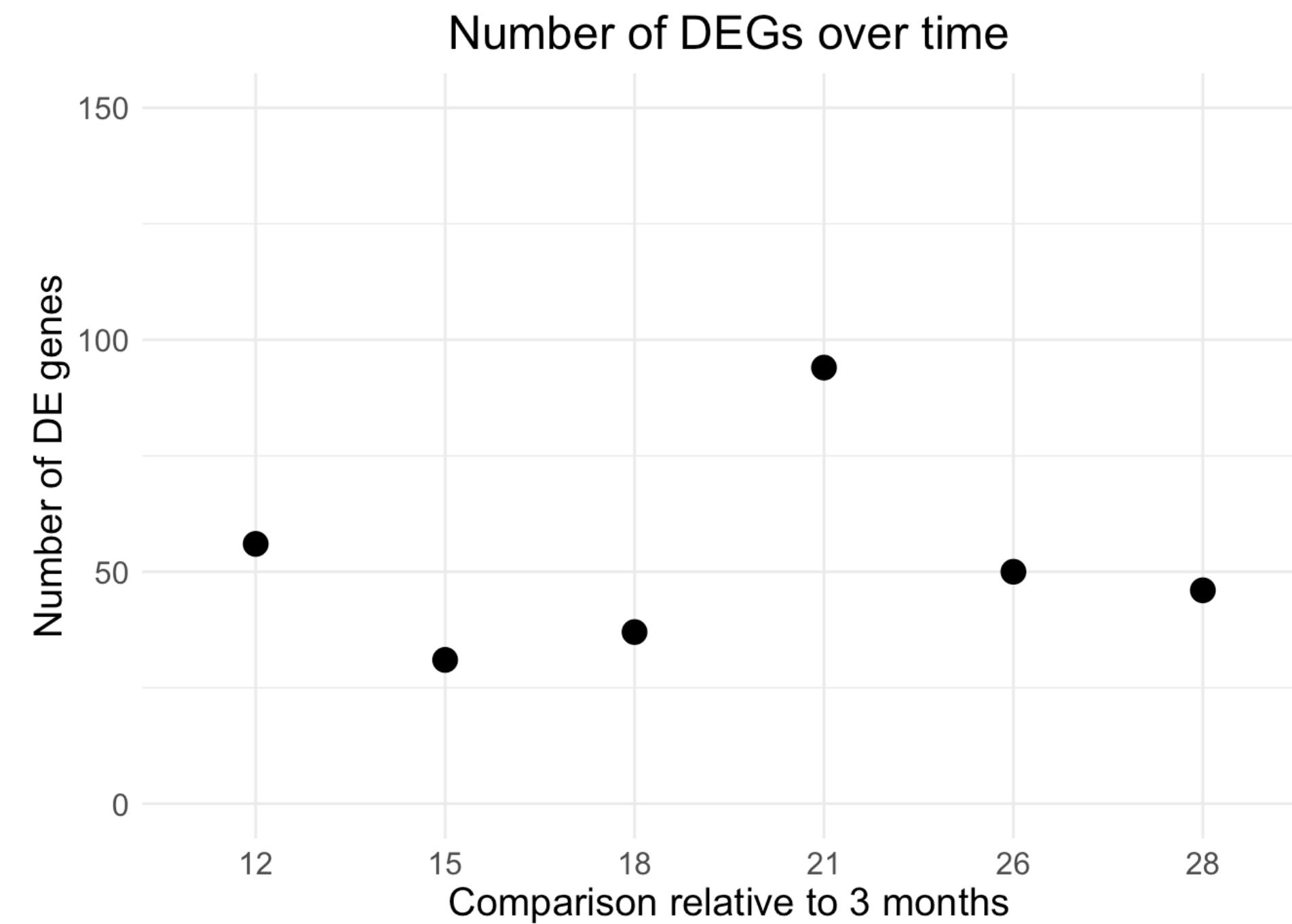
DE Analysis

edgeR - discrete of time points



DE Analysis

edgeR - discrete of time points



Number of DE genes maximizes until around 21 months and then decrease again slightly

Gene Enrichment

C4b

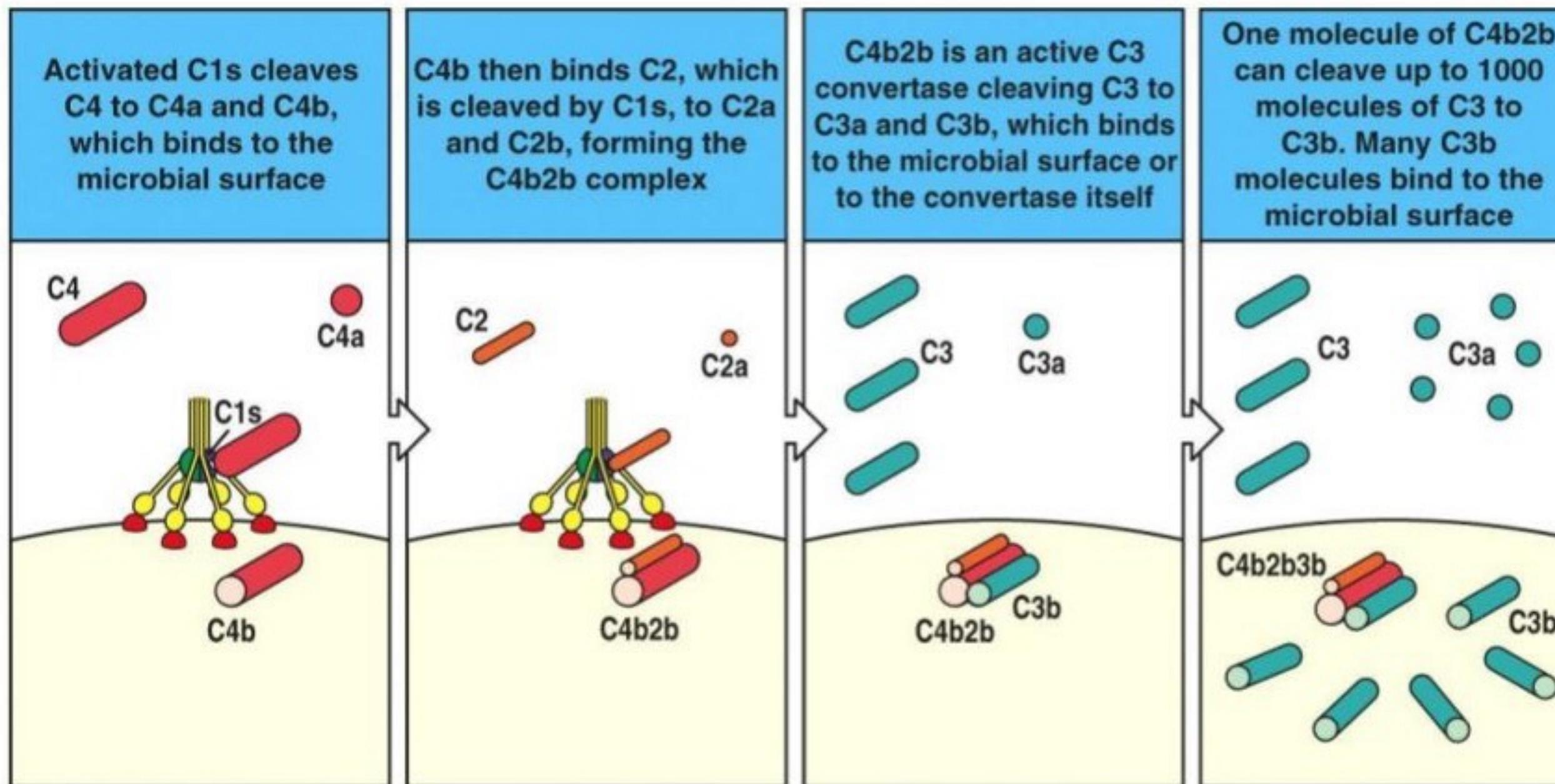


Figure 2-22 Immunobiology, 6/e. (© Garland Science 2005)

[2]

- Part of the classical complement system of the immune system.
- Major risk factor for schizophrenia