Big Data Processing Omics & Bio-Registries - Practical Course

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Objective of the lecture

Practical course on gene expression analysis

Based on a Jupyter notebook

https://jupyter.rwth-aachen.de/

[MMDS_trans] Introduction to Transcriptomics

Cellular Complexity

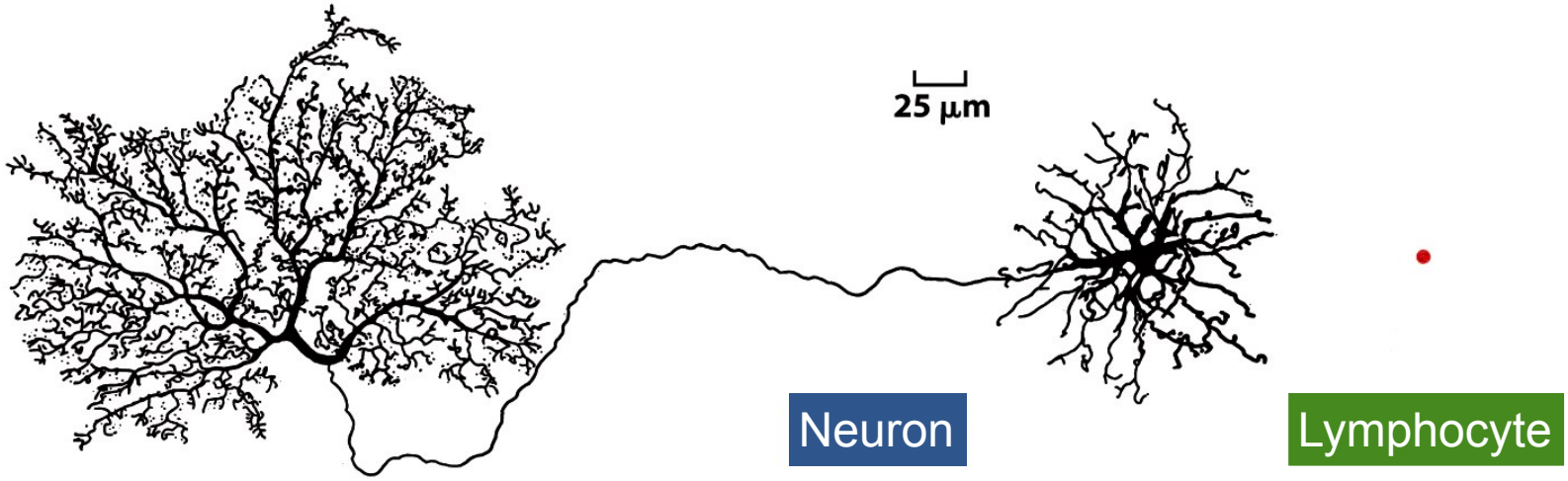


Figure 7-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Two cells of a organism have exactly* the same DNA

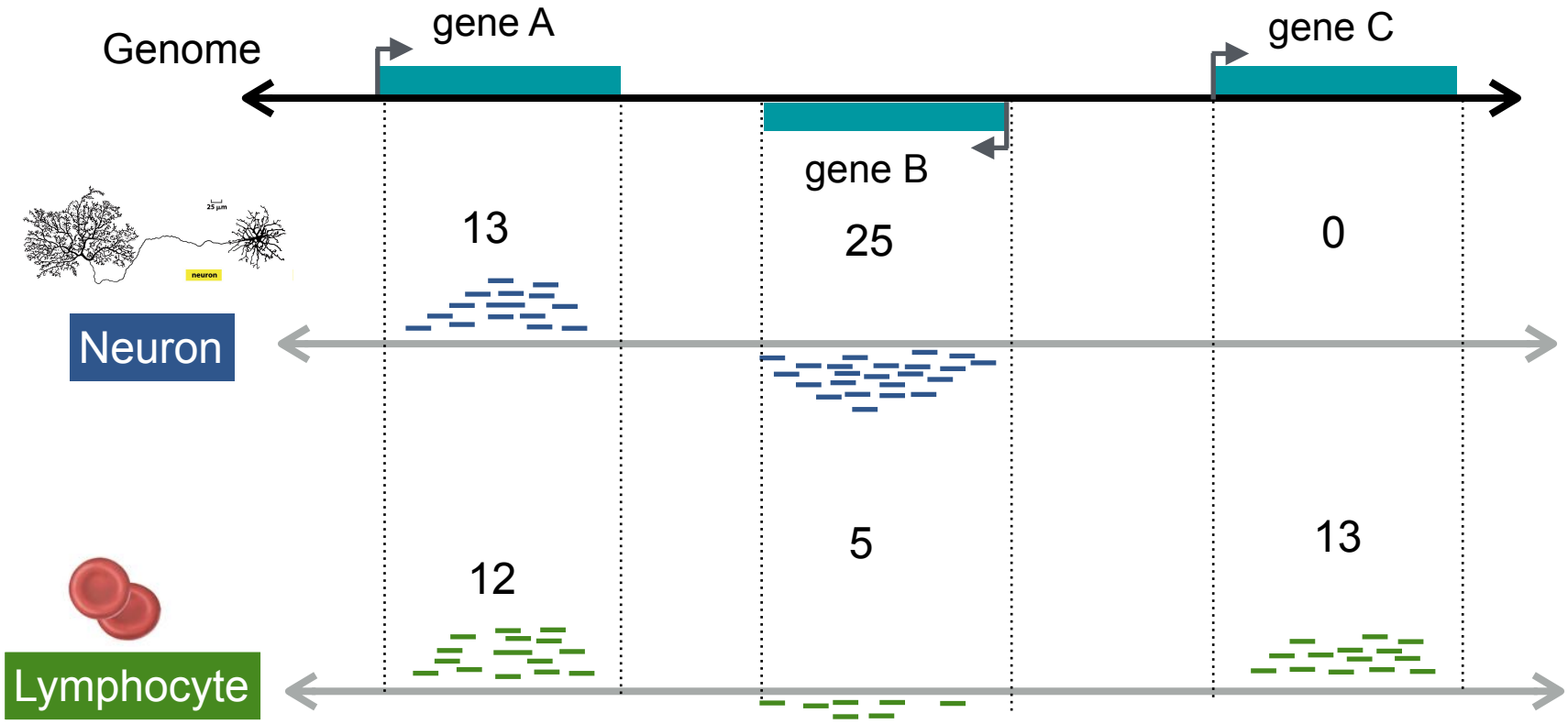
How does this differences arise?

How is cell fate remembered?

*** with exception of somatic mutations and rearrangements of immunological loci**

Gene Quantification

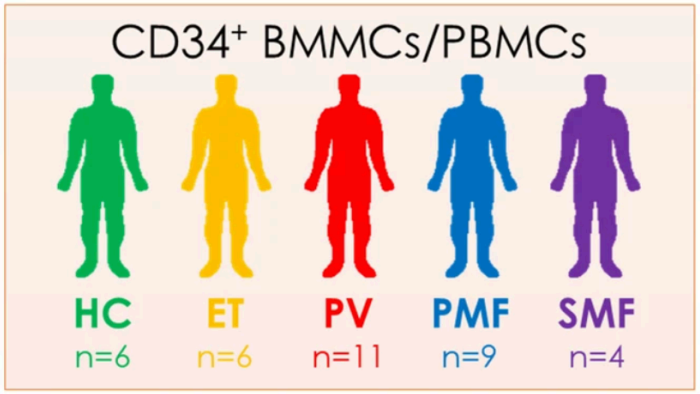
- Perform sequencing for each cell (neuron, lymphocyte)
- Align reads to genome
- Count number of reads inside genes (using known genes annotation)



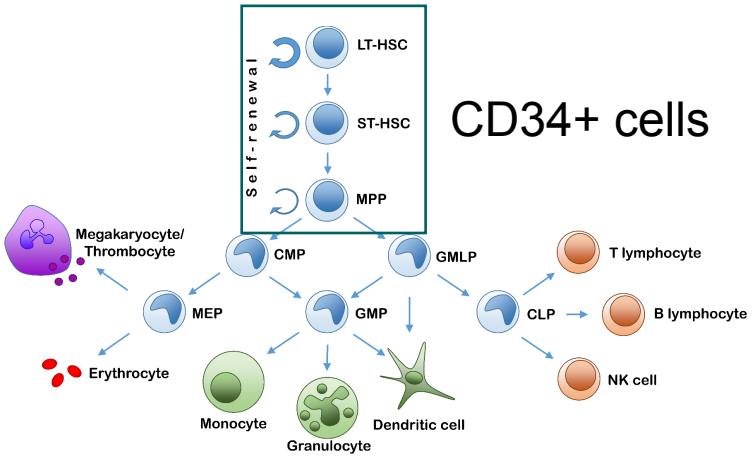
Expression Analysis

- Identify genes related to a particular MPN disease entity
  - example - Baumeister et al. 2021.

- We will consider:
  - Healthy controls (HC)
  - polycythemia vera (PV)
  - essential thrombocythemia (ET)
  - Primary and secondary myelofibrosis (PMF and SMF)

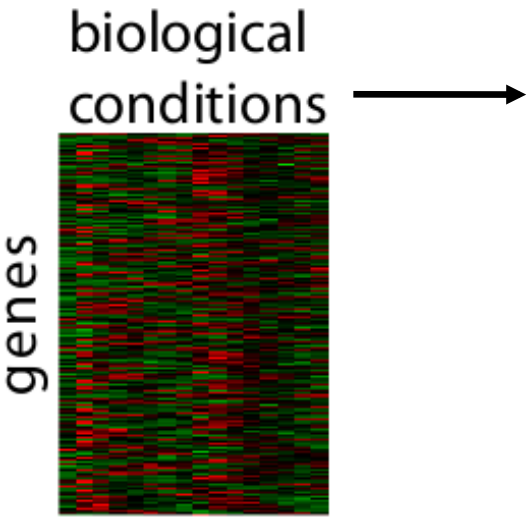


Source: Baumeister et al. 2021.

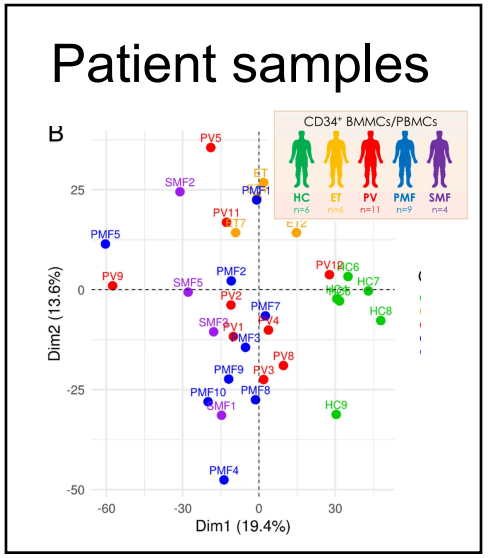


CD34+ cells

Analysis of Gene Expression



gene expression matrix



1- Which genes are up/down regulated after treatment or disease?

- differential analysis / clustering genes

2 - Which cells/patients are more similar?

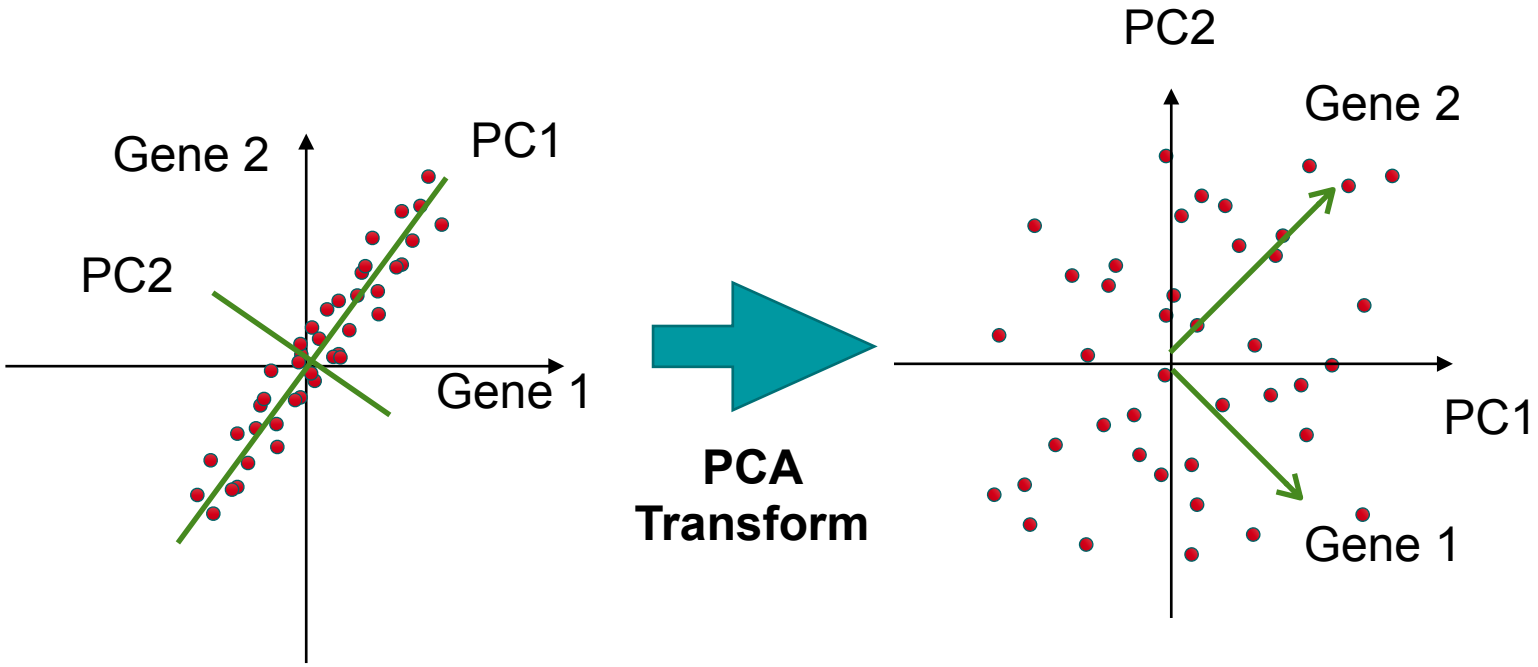
- clustering samples / PCA

3 - How to interpret large lists of genes?

- gene ontology enrichment

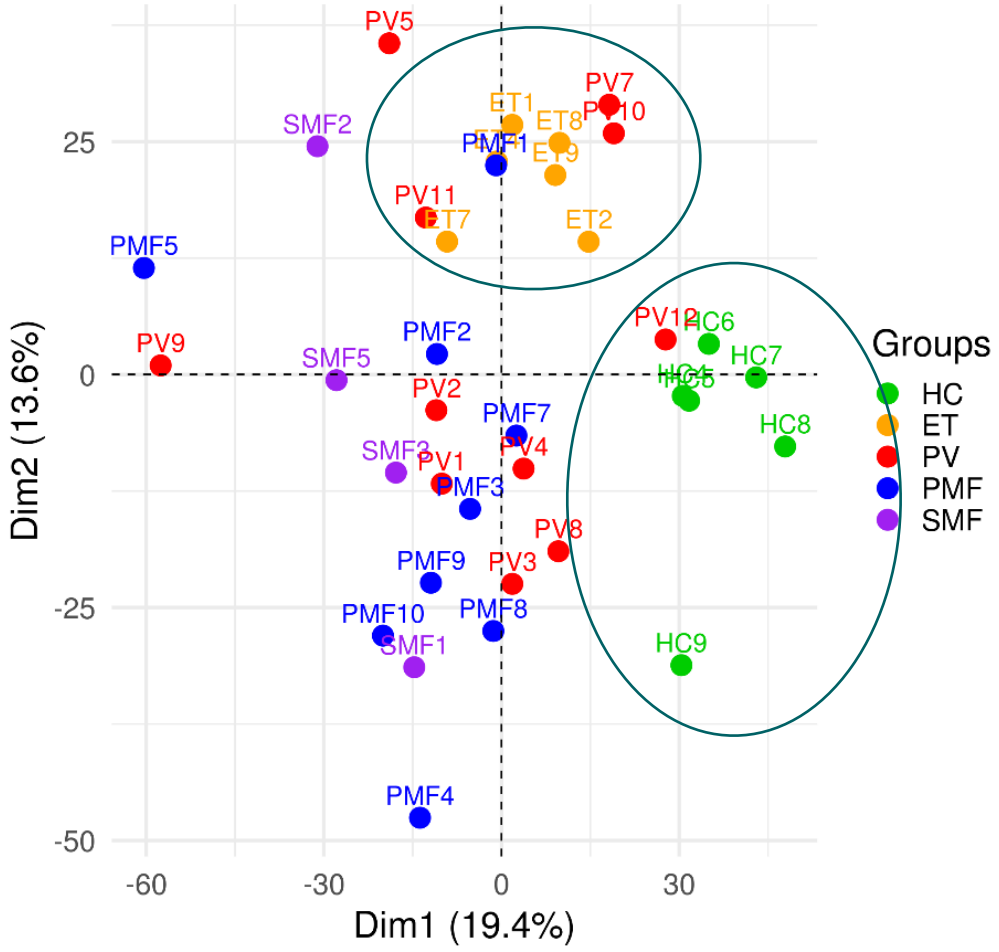
Principal Component Analysis

- **method for dimension reduction**
  - **find combination of genes explaining cells with distinct expression**
- **finding directions with highest variance**



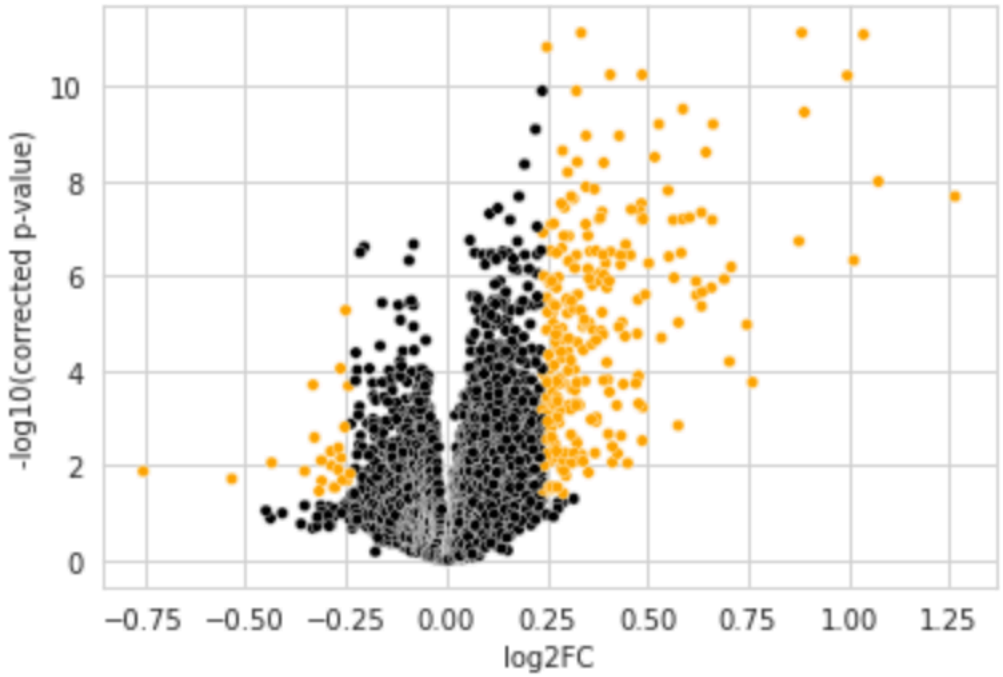
Recommended reading:
Ringner M., *Nature Biotechnology* 26, 303 - 304 (2008)

Gene Expression - PCA Example 2



Differential Gene expression

Volcano Plot - combine p-value and fold change

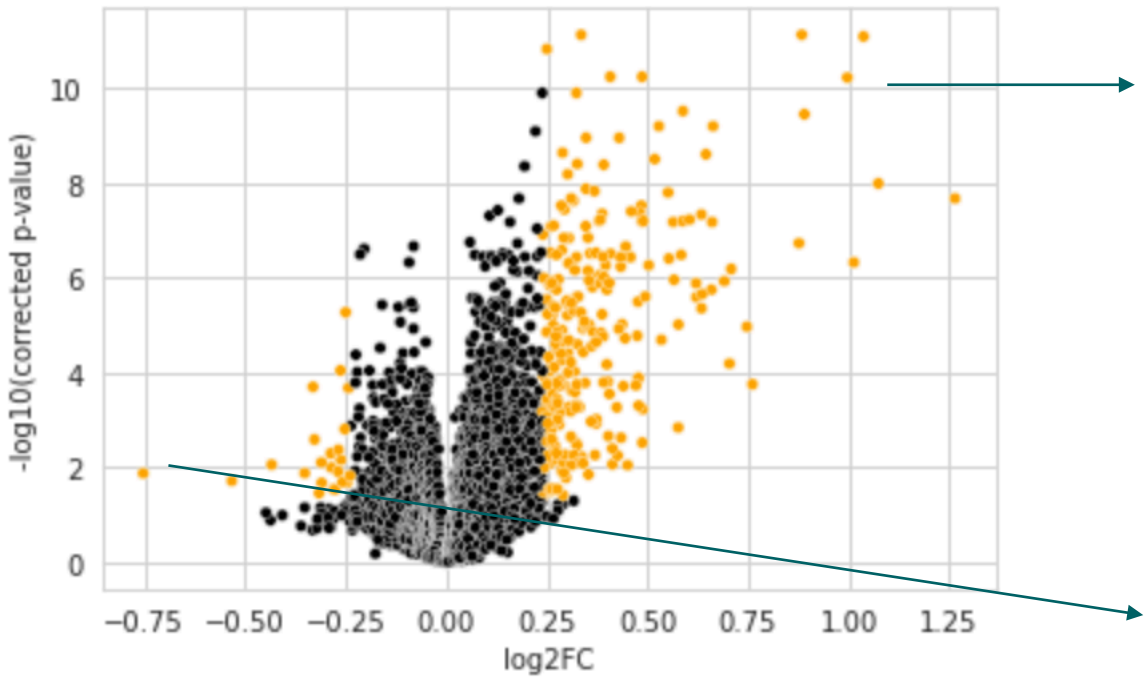


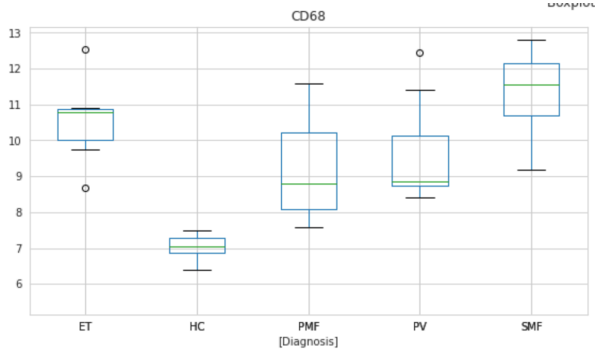
HC genes

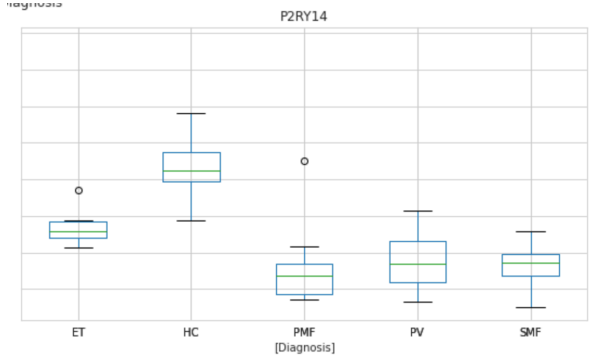
MPN genes

Differential Gene expression

Volcano Plot - combine p-value and fold change



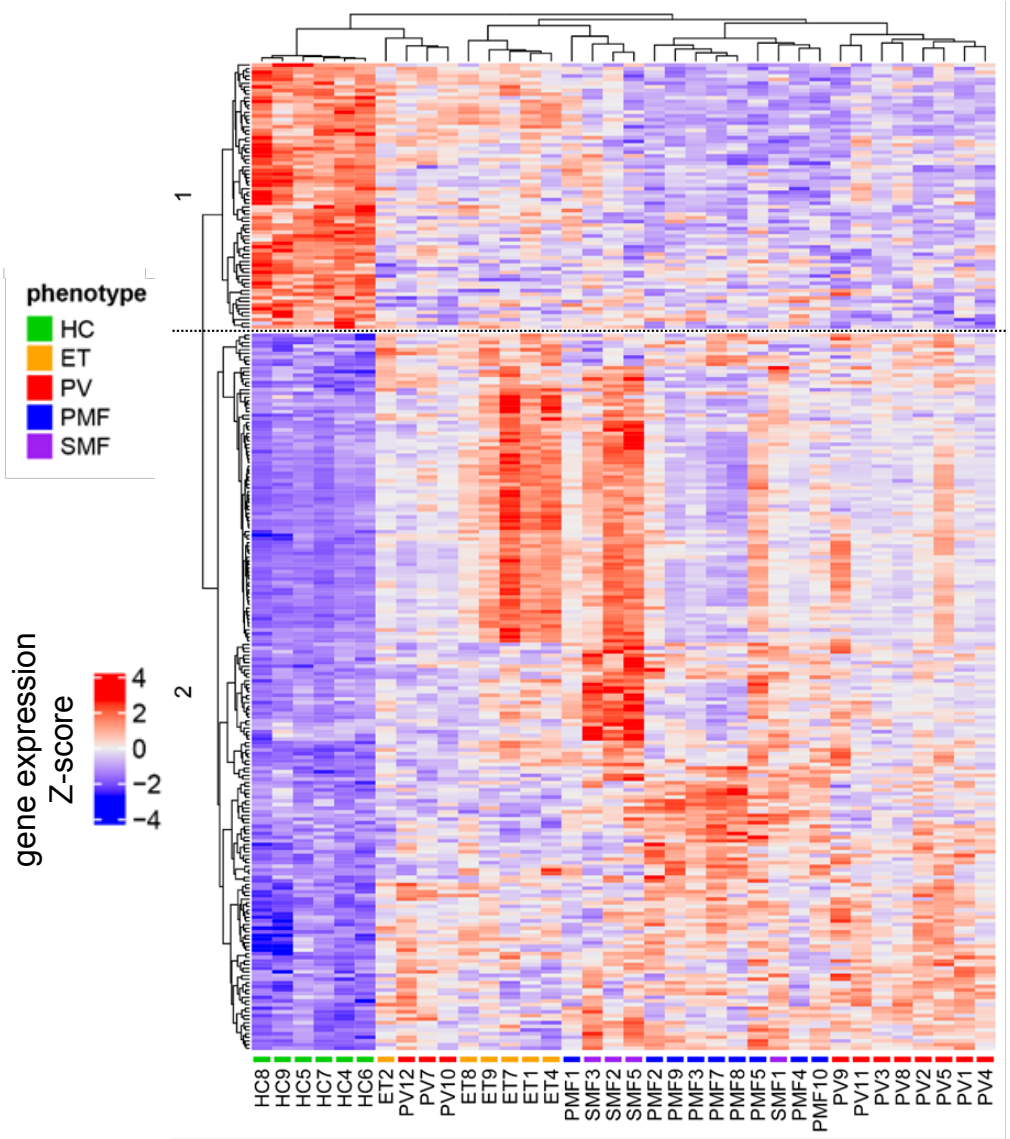




HC genes

MPN genes

Hierarchical Clustering - Average Linkage



- Two clear expression for HC vs. MPNs
- PMF+SMF group together
- Some PV samples group with ET samples

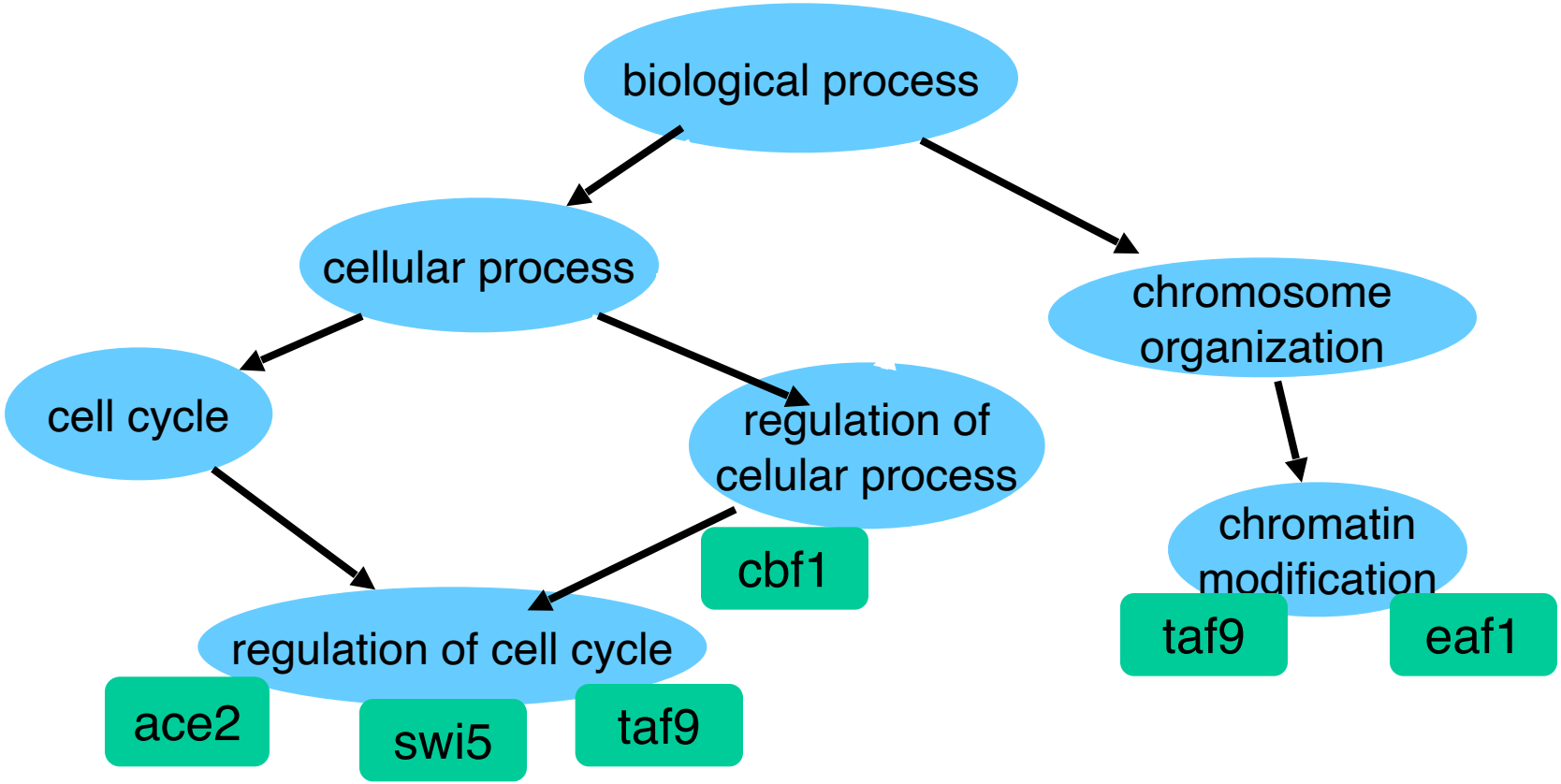
Functional Analysis

Clustering/Differential Expression (DE) returns lists of hundreds of genes How to functionally characterize these?

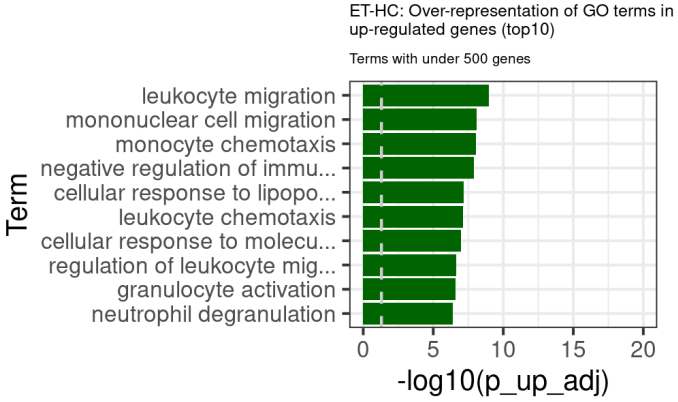
Solution - Relate these genes to annotations from databases

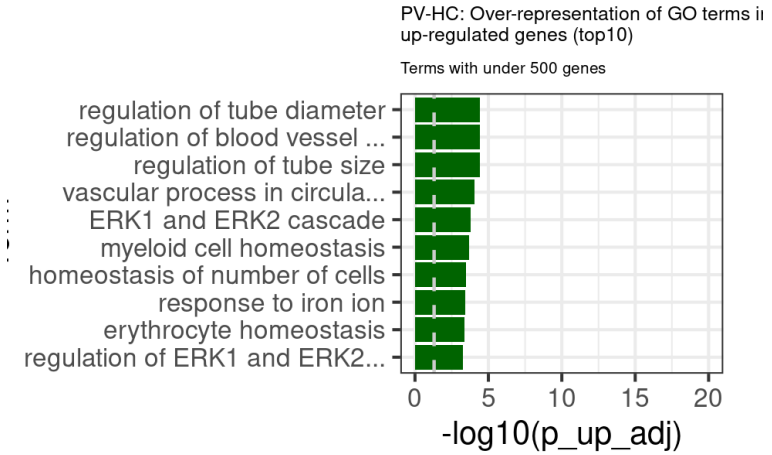
- Gene Ontology, pathways, gene sets, disease ontology, …

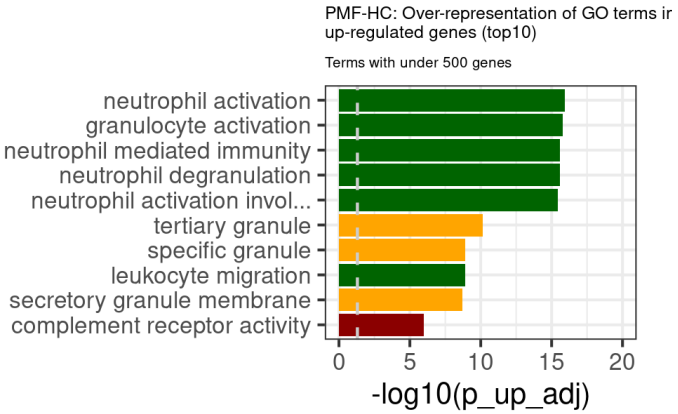
Gene Ontology

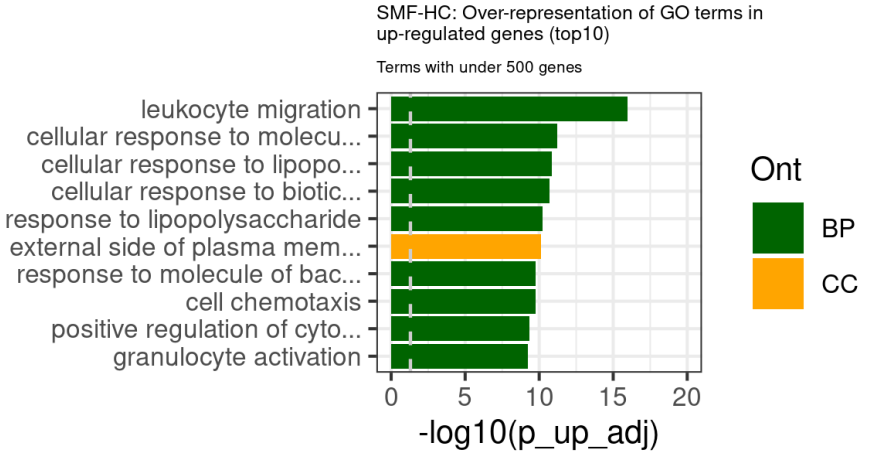


GO Analysis in MPNs









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Resume

- **The course will be done in Jupiter lab**

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