

## Exercise Sheet 06 - Single-cell transcriptomics

Total: 15.0 points

21.06.22 - 28.06.22

### 1. Single-cell technology (1.5 points)

The cell capture rate is the probability that ..

- ☐ ... a droplet has at least one bead
- ☐ ... a droplet has at least one cell

The cell duplication rate is the rate ...

- ☐ ... of cells per droplet
- ☐ ... of barcodes per droplet
- ☐ ... at which captured single cells are associated with two barcodes
- ☒ ... at which captured single cells are associated with two or more different barcodes

UMIs

- ☐ ... are used to identify individual cells
- ☒ ... are used to identify individual molecules

### 2. Barcodes (1.0 + 2.0 bonus points)

- a) What is the minimum barcode length for a synthetic doublet rate of 5% when we assay 1 million cells. (1.0 points)

$4^l$  = number of barcodes of length  $l$   
 $N$  = number of cells,  $M$  = number of barcodes  
5% divergence rate  $\rightarrow M = 20 \cdot N$

$L = \log_4(20 \cdot N)$ , for 1 million cells:  
 $L = \log_4(20 \cdot 1.000.000) = 12.127$

- b) Derive the formula for barcode collisions  $1 - \left(1 - \frac{1}{M}\right)^{N-1}$  shown in the lecture from first principles. (2.0 bonus points)

Hint: you need the probability for picking a barcode  $p = 1/M$  and the binomial coefficient to solve this.

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### 3. Dimensionality reduction (2.5 points, 0.5 each)

The Kullback-Leibler divergence is

- x ... used to measure the difference between two probability distributions
- ... used to measure the difference between data points in low-dimensional space

The first step of t-SNE is to

- ... select neighbours w.r.t. t-distribution over points in high-dimensional space
- x ... select neighbours w.r.t. Gaussian distribution over points in high-dimensional space

The first step of UMAP is to

- ... approximate a manifold in high-dimensional data using principal components
- x ... approximate a manifold in high-dimensional data using simplicial complexes

t-SNE and UMAP are used

- x ... for visualization
- ... as pre-processing
- ... for clustering
- ... for cell type annotation

Which of the following are non-linear method(s)

- x t-SNE
- PCA
- SVD
- x UMAP

#### 4. Seurat (10.0 points)

Seurat is a user-friendly R package for analyzing single-cell data. It's documentation comes with easy-to-follow tutorials: <https://satijalab.org/seurat/index.html>

See this baseline tutorial for more insights, that you will need in this task:

[https://satijalab.org/seurat/articles/pbmc3k\\_tutorial.html](https://satijalab.org/seurat/articles/pbmc3k_tutorial.html)

Install the package and answer the following questions (R markdown files usually work best for workflows like this):

Note: Use the default parameters for all functions, if not stated otherwise.

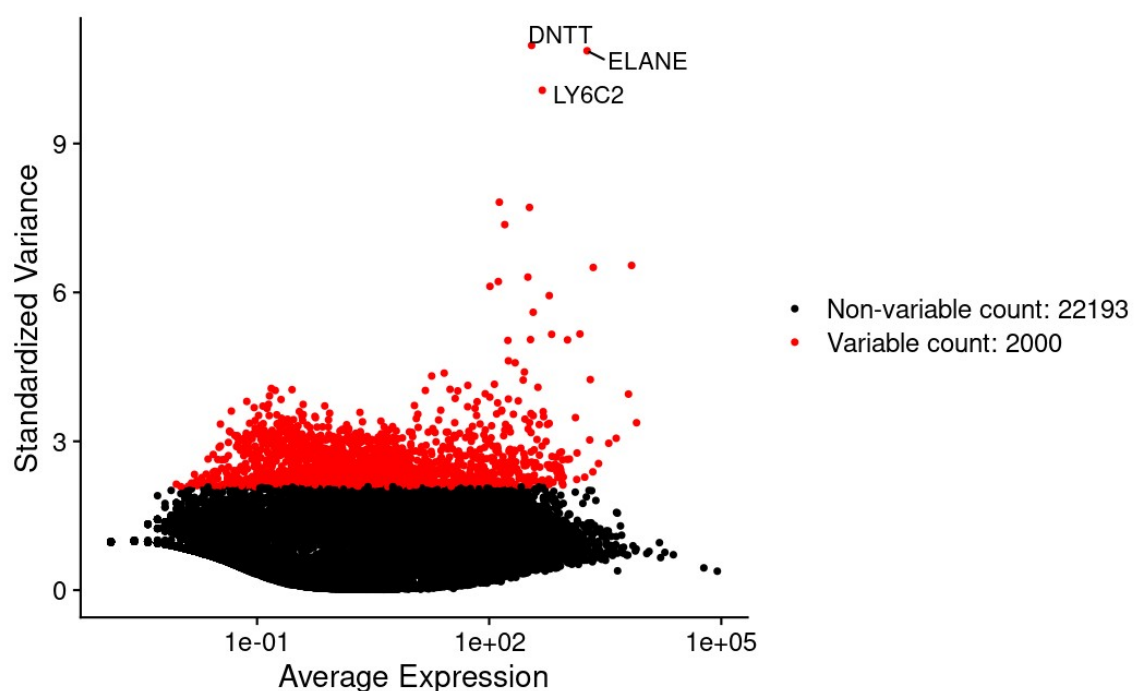
- a) Follow the Cell-Cycle scoring and regression vignette to load and pre-process the data. ([https://satijalab.org/seurat/articles/cell\\_cycle\\_vignette.html](https://satijalab.org/seurat/articles/cell_cycle_vignette.html)). One of the pre-processing steps contains the detection of variable features; explain what this step is doing and name the top 3 variable features. Also add a VariableFeaturesPlot where the three features are labeled. (2.0 points)

Note: do not perform the regression yet, you will need the intermediate results of this section later on.

It creates a mean variability plot and identifies outliers on it

DNTT, ELANE, LY6C2

Variable Features Plot:



- b) Perform the out-regression of cell cycle scores as done in the vignette. Why do we want to regress out cell cycle effects? Do you think this is necessarily a good idea? (2.5 points)

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- c) Extend your script to generate a UMAP plot based on the dataset that resulted **in section a)**. Submit the plot with your solution (1.0 points).

- d) Clustering is a vital part in scRNA-seq analysis. Perform Clustering (*FindNeighbors* & *FindClusters*) and color the UMAP from section c) according to the new clusters. Submit the plot with your solution. The second function has a parameter called 'resolution'; explain what it does. Use a resolution of 1 for the final UMAP. (2.0 points)

- e) Find the differentially expressed markers between all clusters (*FindAllClusters*). Can you find any of the cell-cycle marker genes in the results? Find a cluster, in which marker genes for the G2M cycle are differentially expressed, name the cluster, the genes (2 are enough) and the corresponding log2 Fold-change. (2.5 points)

Hint: You find the maker genes in the lists that are created in the first code-block of the cell-cycle scoring vignette (*g2m.genes*)

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