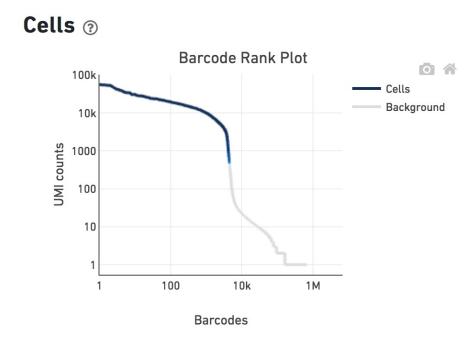
Single cell transcriptomics

Quality control

Cell calling

Dropouts: low #UMI/cell



Further cell filtering

- Doublets: DoubletFinder
- % UMI in mitochondrial genes:
 - Points to dying/stressed cells
 - Filter by threshold (e.g. 20%)
- Number of detected genes/cell
 - · High: possible doublet
 - Low: possible dropout

McGinnis CS et al. DoubletFinder: Doublet Detection in Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. Cell Syst; 2019;8:329-337

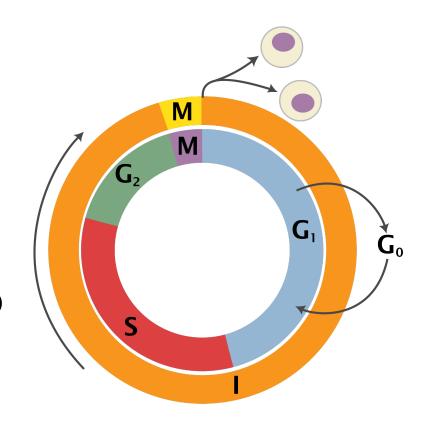
Confoundig variables

- % mitochondria
- % ribosomal genes
- % dissociation genes
- Library size
- Sex
- Age
- Cell cycle

• . .

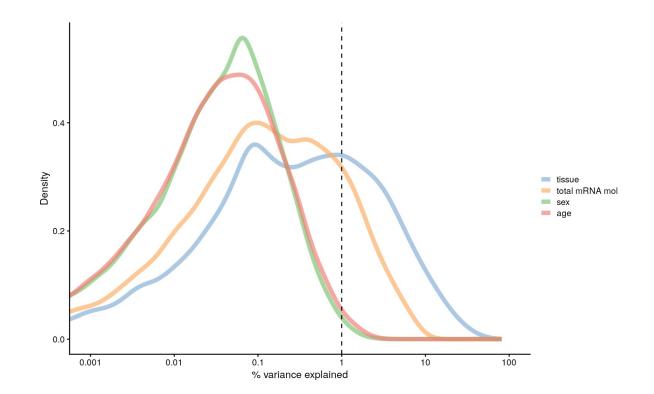
Cell cycle

- Default clustering might be according to state
- Cycling state can be regressed out for clustering according to cell type



Find explanatory variables

- With the package scater
- Plot variance explained per gene



Regress out for clustering

- Regress out variables explaining a lot of variation
- At the scaling step -> affects PCA and therefore clustering/annotation

• OR: integrate data (day 2)