Look more into fixed versus adaptive thresholds for QC: http://bioconductor.org/books/3.13/OSCA.basic/quality-control.html#identifying-low-quality-cells

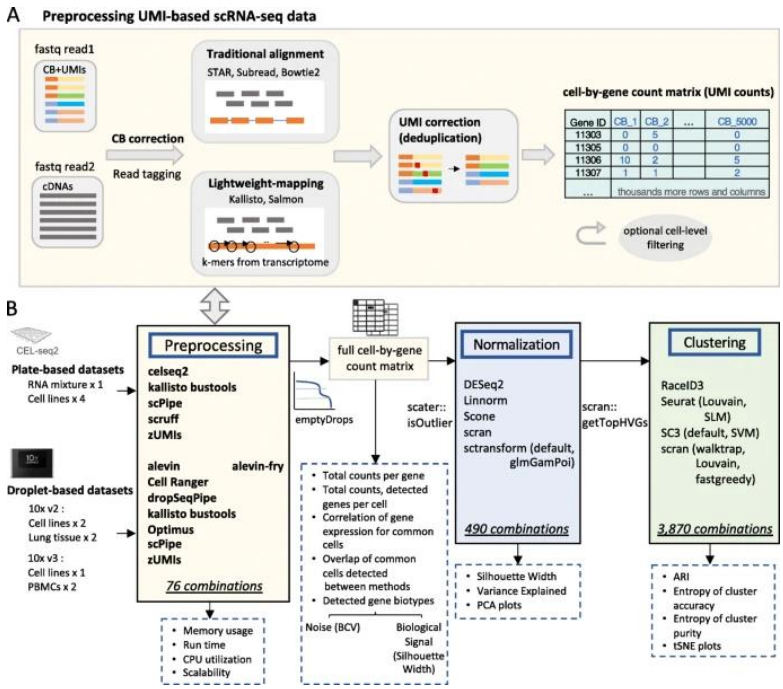
https://doi.org/10.1186/s13059-020-02136-7 -> practical recommendations, also interesting plots in supplementary

Performance Assessment and Selection of Normalization Procedures for Single-Cell RNA-Seq: https://www.bioconductor.org/packages/release/bioc/vignettes/scone/inst/doc/sconeTutorial.html

Current best practices in single-cell RNA-seq analysis: a tutorial -> There is no consensus on scaling genes to 0 mean and unit variance. We prefer not to scale gene expression

Diagnostic plots for filtering, normalization, feature selection:

Try a simply normalization technique like log1pPF and assess normalization result by visualizing it on a UMAP with respect to total counts and highly expressed genes in your dataset.



https://doi.org/10.1186/s13059-021-02552-3

https://www.sc-best-practices.org/preprocessing_visualization/normalization.html

"We cannot treat all genes the same": https://hbctraining.github.io/scRNA-seq_online/lessons/06_SC_SCT_normalization.html

https://htmlpreview.github.io/?https://github.com/satijalab/sctransform/blob/supp_html/supplement/variance_stabilizing_transformation.html

PCA: https://hbctraining.github.io/scRNA-seq_online/lessons/05_theory_of_PCA.html

Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression, https://doi.org/10.1186/s13059-019-1874-1, https://www.biorxiv.org/content/10.1101/2021.07.07.451498v1

"For reference, we first apply the standard Seurat workflow, with log-normalization"

“For comparison, we now apply sctransform normalization” # Note that this single command replaces NormalizeData, ScaleData, and # FindVariableFeatures.

https://htmlpreview.github.io/?https://github.com/satijalab/sctransform/blob/supp_html/supplement/seurat.html

Implementation: https://satijalab.org/seurat/articles/sctransform_v2_vignette.html

pipeComp, a general framework for the evaluation of computational pipelines, reveals performant single cell RNA-seq preprocessing tools, https://doi.org/10.1186/s13059-020-02136-7

-> We might want to keep more features if we can go on PSB cluster (up to 4000)

https://www.sc-best-practices.org/cellular_structure/annotation.html

Manual annotation versus other methods:

For manual annotation: get marker genes from https://training.galaxyproject.org/training-material/topics/single-cell/tutorials/scrna-plant/tutorial.html

Tutorial: https://hbctraining.github.io/scRNA-seq_online/lessons/09_merged_SC_marker_identification.html

https://www.sciencedirect.com/science/article/pii/S2001037021000192

http://bioconductor.org/books/release/SingleRBook/introduction.html

https://github.com/Irrationone/cellassign

https://docs.scvi-tools.org/en/stable/tutorials/index.html

Integration: https://satijalab.org/seurat/articles/sctransform_v2_vignette.html

From Louvain to Leiden: guaranteeing well-connected communities

Bias, robustness and scalability in single-cell differential expression analysis