

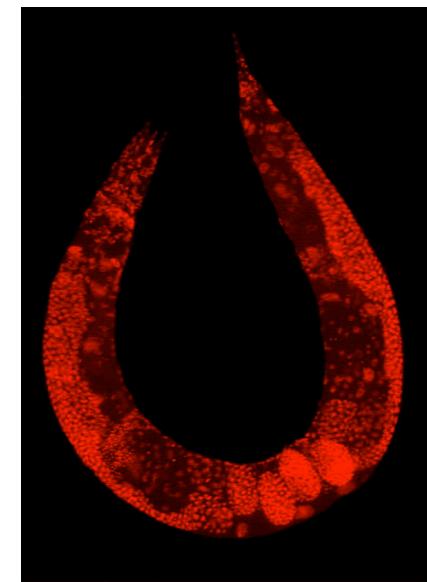
Swiss Institute of  
Bioinformatics

# Single cell transcriptomics data analysis Day 2

## Cell type annotation

# What is a “cell type”?

- Fundamental unit of life
- Originally defined in terms of function, location tissue type, cell morphology
- Later extended to
  - presence/absence of cell surface markers
  - gene expression (molecular profile)
- Currently very much less fixed
  - cell cycle phase
  - migration state
  - differentiation: cell state

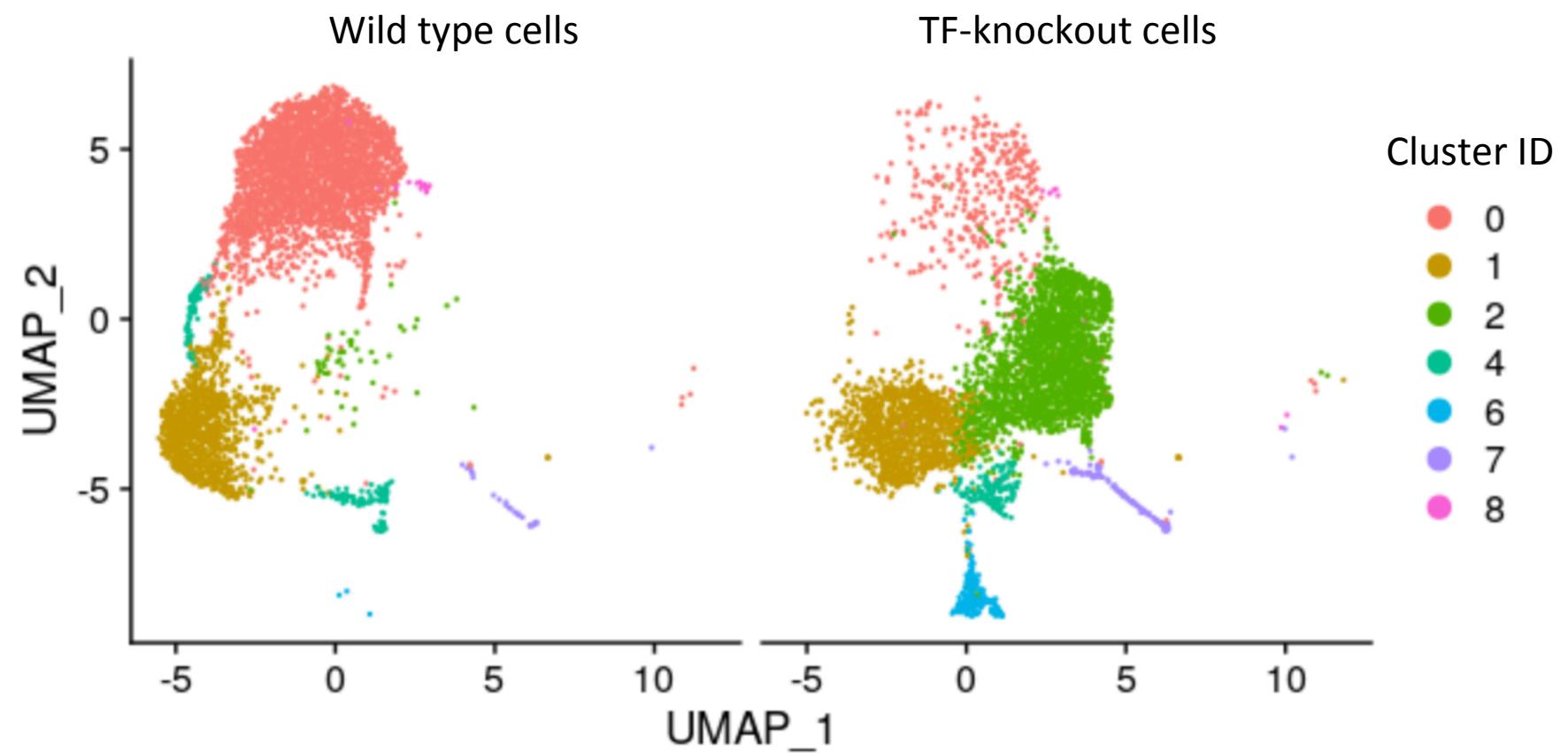


Wild-type *C. elegans* hermaphrodite  
stained to highlight the nuclei  
of all cells

# Why should we identify cell types?

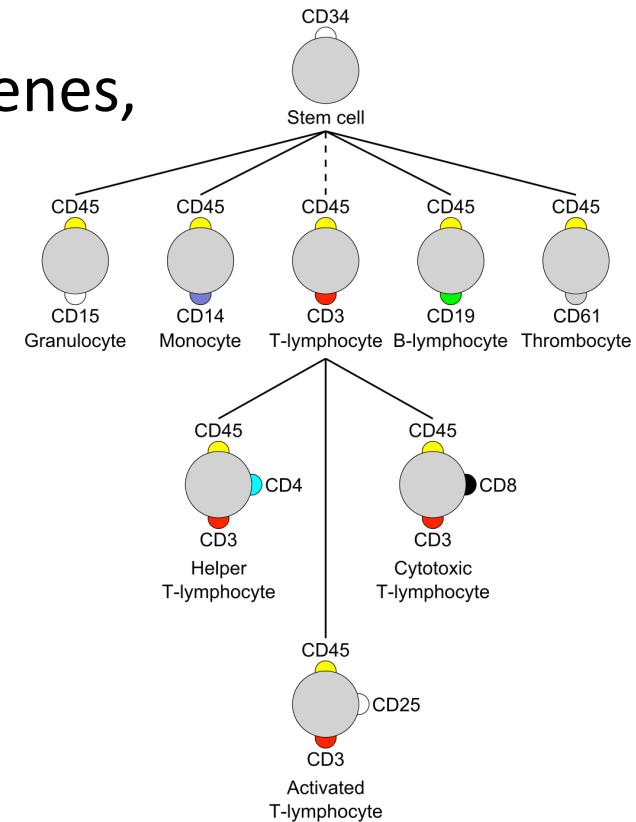
- Samples are heterogeneous (in general)
- Tumor sample: how much do they differ from normal cell types?
- Find new cell types which have been missed by using “standard” surface markers
- To compare the abundance of cell types in different conditions
- Follow cell fate and determine cell differentiation mechanisms
- To determine which cell types might communicate with each other
- ...

# Change in cell type abundances: what are the new cells?



# Cell surface markers

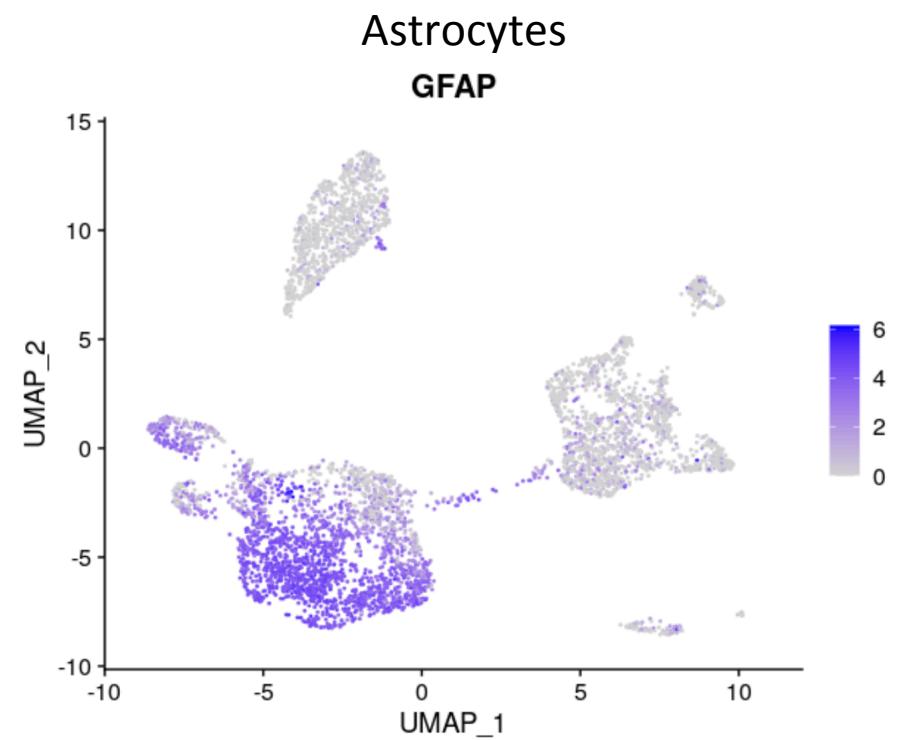
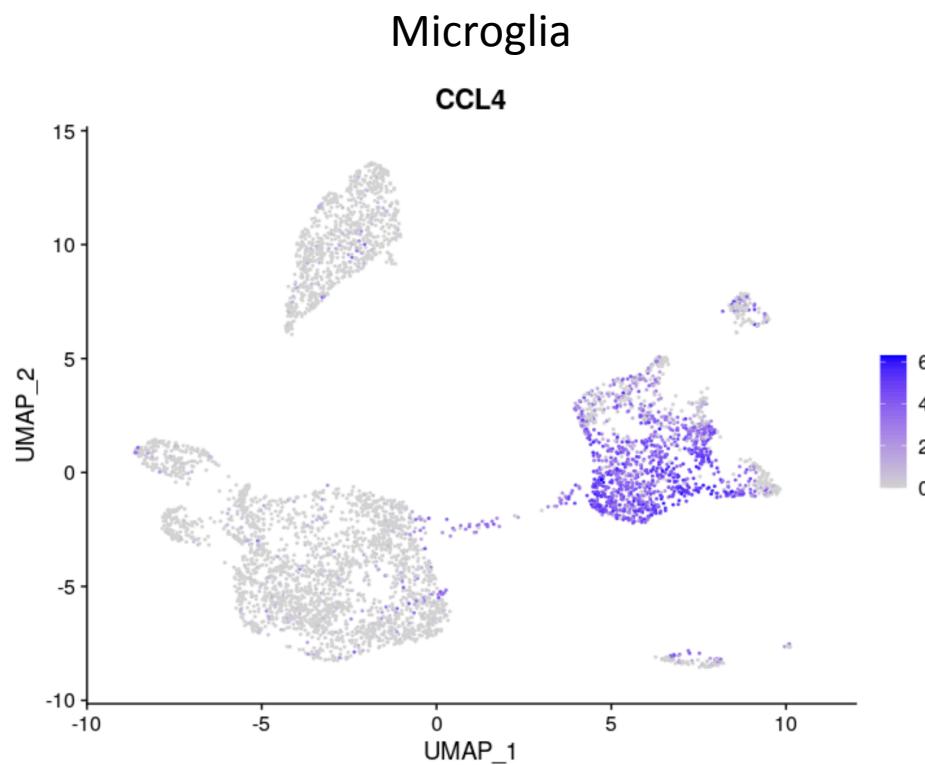
- Often considered the gold standards esp. in immunology
- mRNA of cell surface markers sometimes lowly expressed or absent
- Use a combination of such markers genes, and also other genes like DE genes between one cluster compared to the other clusters (eg transcription factors)



# Manual vs automatic cell type annotation

- Manual: **using marker genes**
  - What most people do...
  - Time consuming
  - Requires expert knowledge
  - Sometimes subjective and inaccurate
- Automatic: **requires a reference**
  - Use complete cell type-specific mRNA expression profiles based on bulk RNAseq from FACS-sorted 'pure' populations
  - OR: Use "a reference" of manually curated cells picked from scRNA-seq data sets
  - Can miss cell types if they are not included in the reference
- Methods:
  - Assign a cell type per individual cell or per cluster of cells (better per cell)
  - Assignment of cell type via correlation of each cell/cluster to the "reference"

# Manual annotation using known marker genes



Human glioblastoma multiforme cells, 10x Genomics data

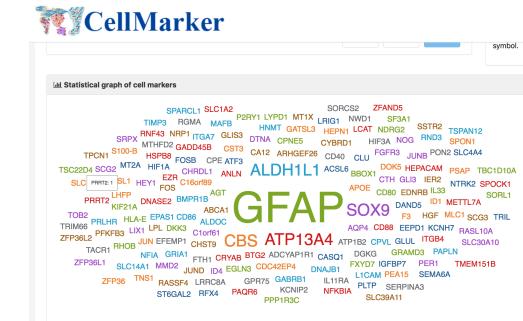
[https://support.10xgenomics.com/single-cell-gene-expression/datasets/4.0.0/Parent\\_SC3v3\\_Human\\_Glioblastoma](https://support.10xgenomics.com/single-cell-gene-expression/datasets/4.0.0/Parent_SC3v3_Human_Glioblastoma)

# Databases with cell type marker genes

- PanglaoDB <https://panglaodb.se/> (mouse and human)

- CellMarker (mouse and human)

<http://biocc.hrbmu.edu.cn/CellMarker/>

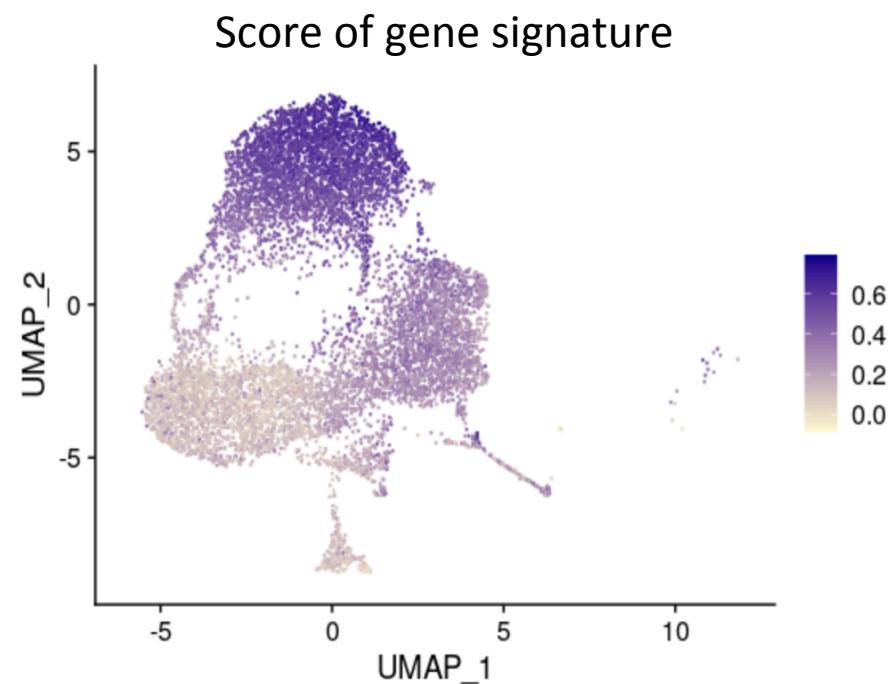


- SingleR <https://github.com/dviraran/SingleR> (Aran et al. 2019), access via celldex package, eg human primary cell atlas (microarrays)
  - Human Cell Atlas <https://www.humancellatlas.org> (Regev et al) single cell RNA seq atlas, also some mouse data

# Module score

Tirosh et al 2016, Science 352:6282

Compare expression level of genes belonging to the signature to “control” genes with similar expression level to signature genes



# SingleR

## SingleR

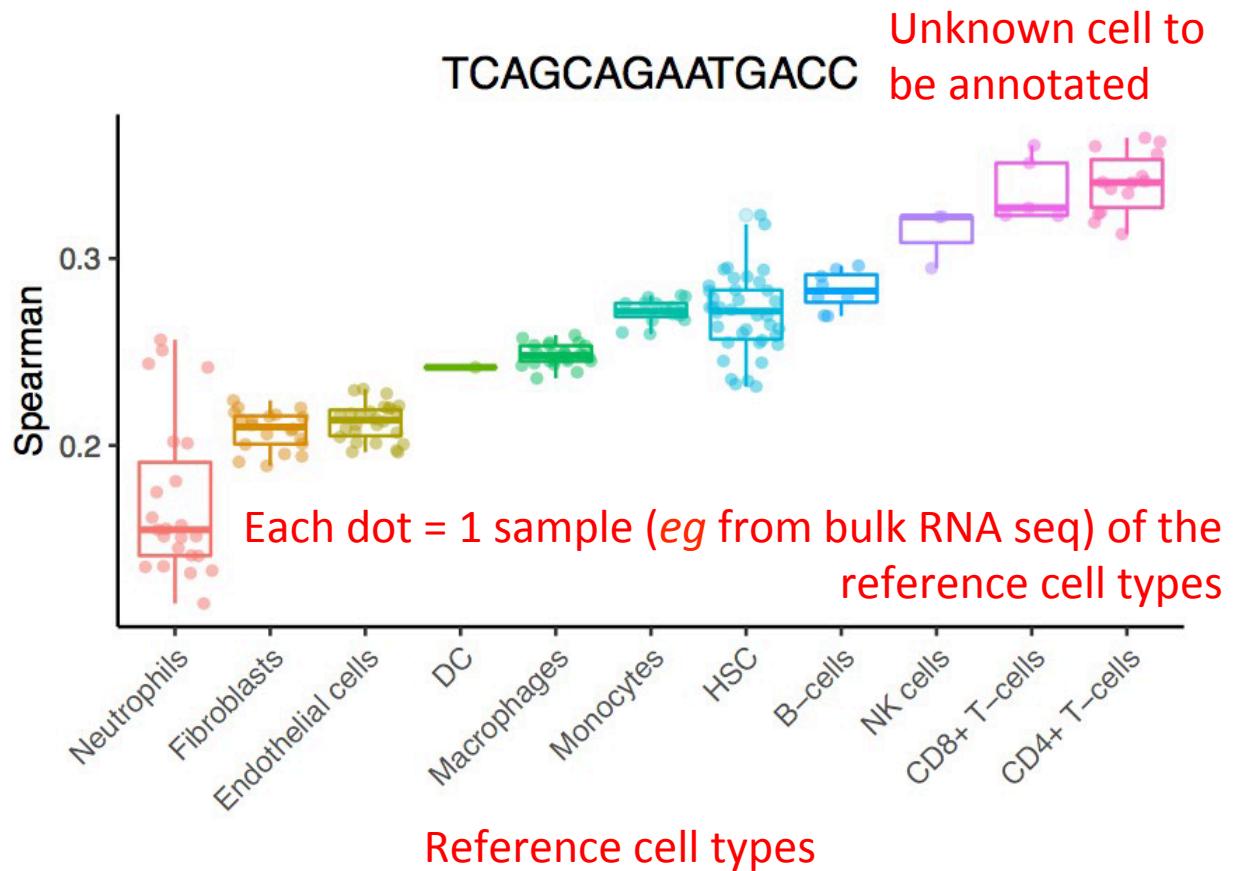
Easy access to rich reference data:

- HPCA: hand-annotated Human Primary Cell Atlas  
37 main types, 157 subtypes, 713 samples
- BluePrint +ENCODE  
24 main types, 43 subtypes, 259 bulk RNAseq samples
- Mouse: ImmGen and ‘mouse.rnaseq’ (brain-specific)

Classifies cells to both main types and subtypes,  
performs both single cell-wise and cluster-wise  
annotation

# SingleR

Correlate each cell (or average of cluster) to each reference cell type

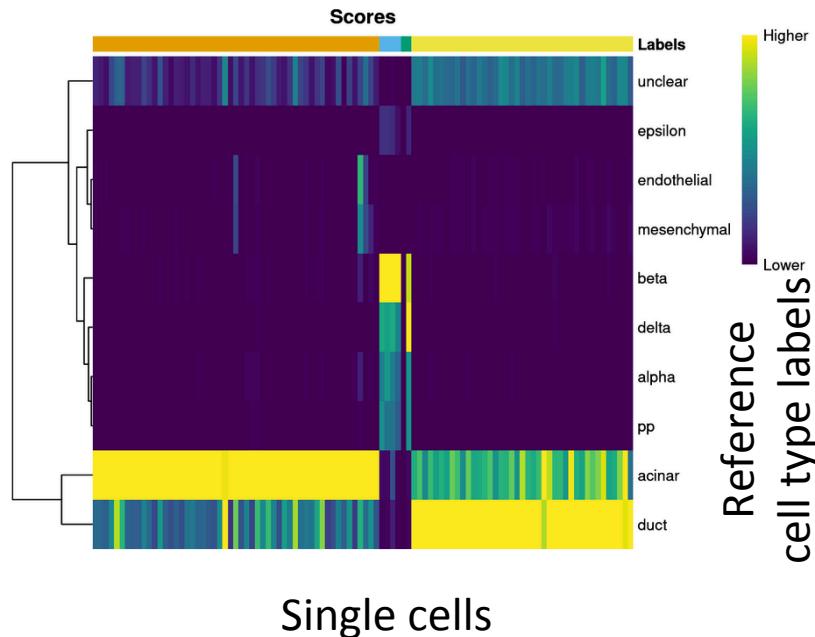


Per reference cell type, correlate to the topN genes that have  $\text{median(expression)} > \text{median(expression)}$  in all other cell types

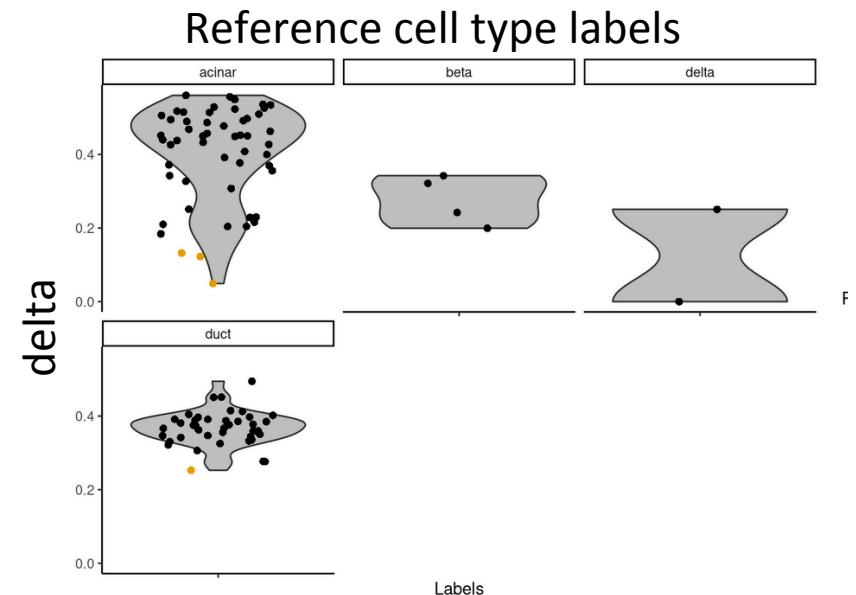
Fine Tuning step...

# SingleR – annotation diagnostics

Heatmap of scores per cell for each label:



```
plotScoreHeatmap(singleR.object)
```

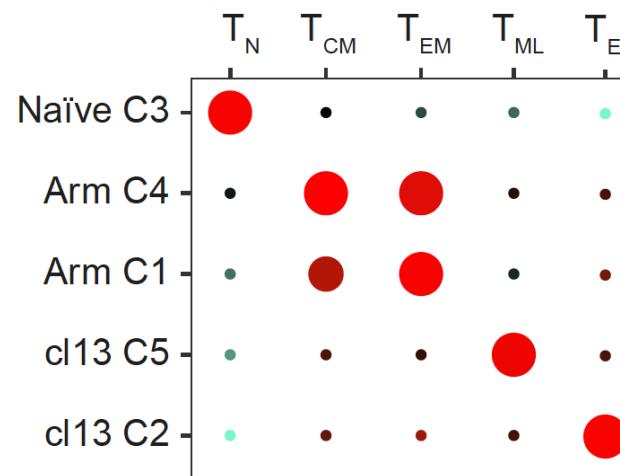
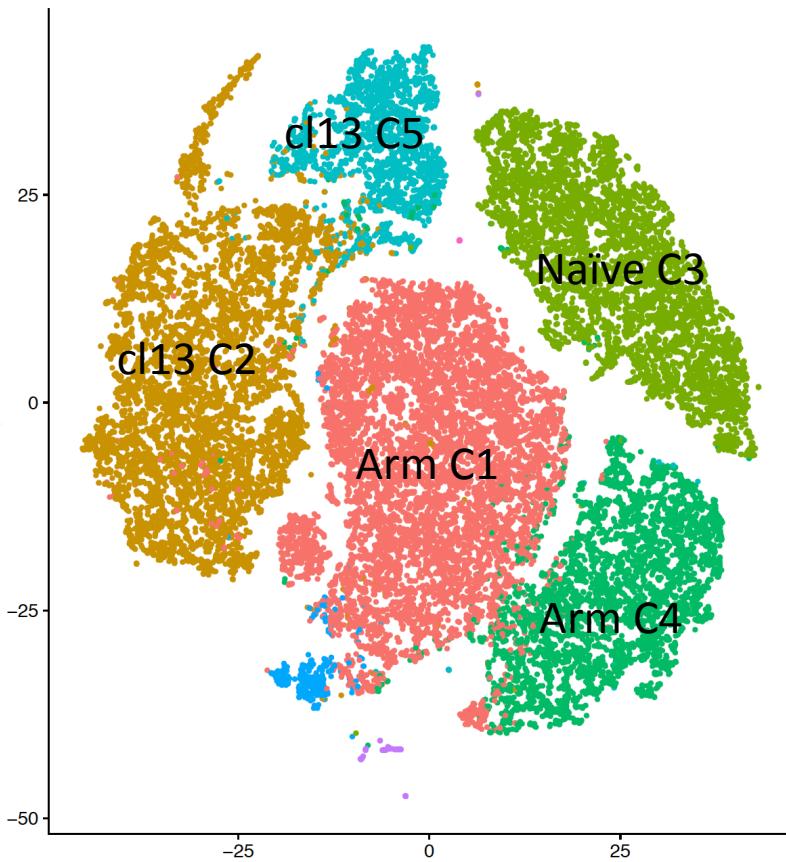


```
plotDeltaDistribution(singleR.object)
```

Try these during the practical exercise!

[https://bioconductor.org/packages/release/bioc/vignettes/SingleR/inst/doc/SingleR.html#4\\_Annotation\\_diagnostics](https://bioconductor.org/packages/release/bioc/vignettes/SingleR/inst/doc/SingleR.html#4_Annotation_diagnostics)

# SingleR can also be used to evaluate similarity to specific types



Arm and cl13 = 2 different strains of LCM virus

Charmoy et al 2021 <https://doi.org/10.1016/j.celrep.2021.109672>

# Several methods are available

**Table 1 Automatic cell identification methods included in this study**

From: [A comparison of automatic cell identification methods for single-cell RNA sequencing data](#)

Name	Version	Language	Underlying classifier	Prior knowledge	Rejection option	Reference
Garnett	0.1.4	R	Generalized linear model	Yes	Yes	[14]
Moana	0.1.1	Python	SVM with linear kernel	Yes	No	[15]
DigitalCellSorter	GitHub version: e369a34	Python	Voting based on cell type markers	Yes	No	[16]
SCINA	1.1.0	R	Bimodal distribution fitting for marker genes	Yes	No	[17]
scVI	0.3.0	Python	Neural network	No	No	[18]
Cell-BLAST	0.1.2	Python	Cell-to-cell similarity	No	Yes	[19]
ACTINN	GitHub version: 563bcc1	Python	Neural network	No	No	[20]
LAmbDA	GitHub version: 3891d72	Python	Random forest	No	No	[21]
scmapcluster	1.5.1	R	Nearest median classifier	No	Yes	[22]
scmapcell	1.5.1	R	kNN	No	Yes	[22]
scPred	0.0.0.9000	R	SVM with radial kernel	No	Yes	[23]
CHETAH	0.99.5	R	Correlation to training set	No	Yes	[24]
CaSTLe	GitHub version: 258b278	R	Random forest	No	No	[25]
SingleR	0.2.2	R	Correlation to training set	No	No	[26]
sclD	0.0.0.9000	R	LDA	No	Yes	[27]
singleCellNet	0.1.0	R	Random forest	No	No	[28]
LDA	0.19.2	Python	LDA	No	No	[29]
NMC	0.19.2	Python	NMC	No	No	[29]
RF	0.19.2	Python	RF (50 trees)	No	No	[29]
SVM	0.19.2	Python	SVM (linear kernel)	No	No	[29]
SVM <sub>rejection</sub>	0.19.2	Python	SVM (linear kernel)	No	Yes	[29]
kNN	0.19.2	Python	kNN ( $k = 9$ )	No	No	[29]

# Seurat, Azimuth

## Cell type label transfer



## Mapping and annotating query datasets

Compiled: 2021-06-14

Source: vignettes/integration\_mapping.Rmd

## Introduction to single-cell reference mapping

In this vignette, we first build an integrated reference and then demonstrate how to leverage this reference to annotate new query datasets. Generating an integrated reference follows the same workflow described in more detail in the integration introduction vignette. Once generated, this reference can be used to analyze additional query datasets through tasks like cell type label transfer and projecting query cells onto reference UMAPs. Notably, this does not require correction of the underlying raw query data and can therefore be an efficient strategy if a high quality reference is available.

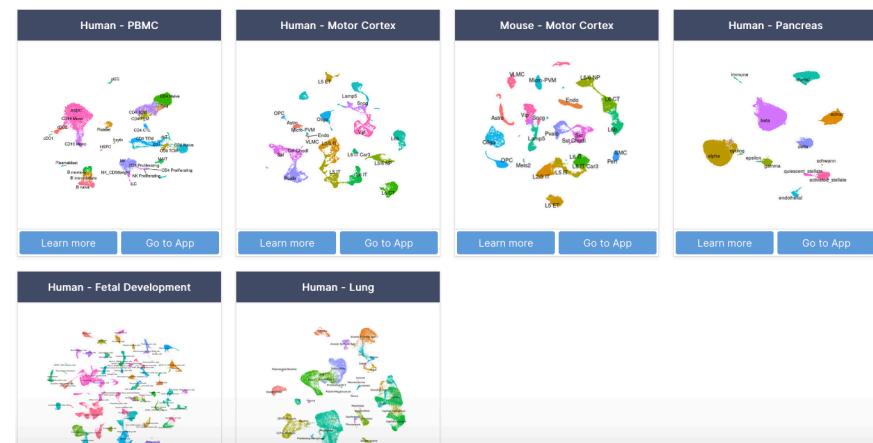
[https://satijalab.org/seurat/articles/integration\\_mapping.html](https://satijalab.org/seurat/articles/integration_mapping.html)

## Reference-based mapping

Azimuth is a web application for reference-based single-cell analysis. It uses an annotated reference dataset to automate processing, analysis, and interpretation of new single-cell RNA-seq experiments. Azimuth leverages a 'reference-based mapping' pipeline that inputs a counts matrix of gene expression in single cells, and performs normalization, visualization, cell annotation, and differential expression (biomarker discovery). All results can be explored within the app, and easily downloaded for additional downstream analysis.

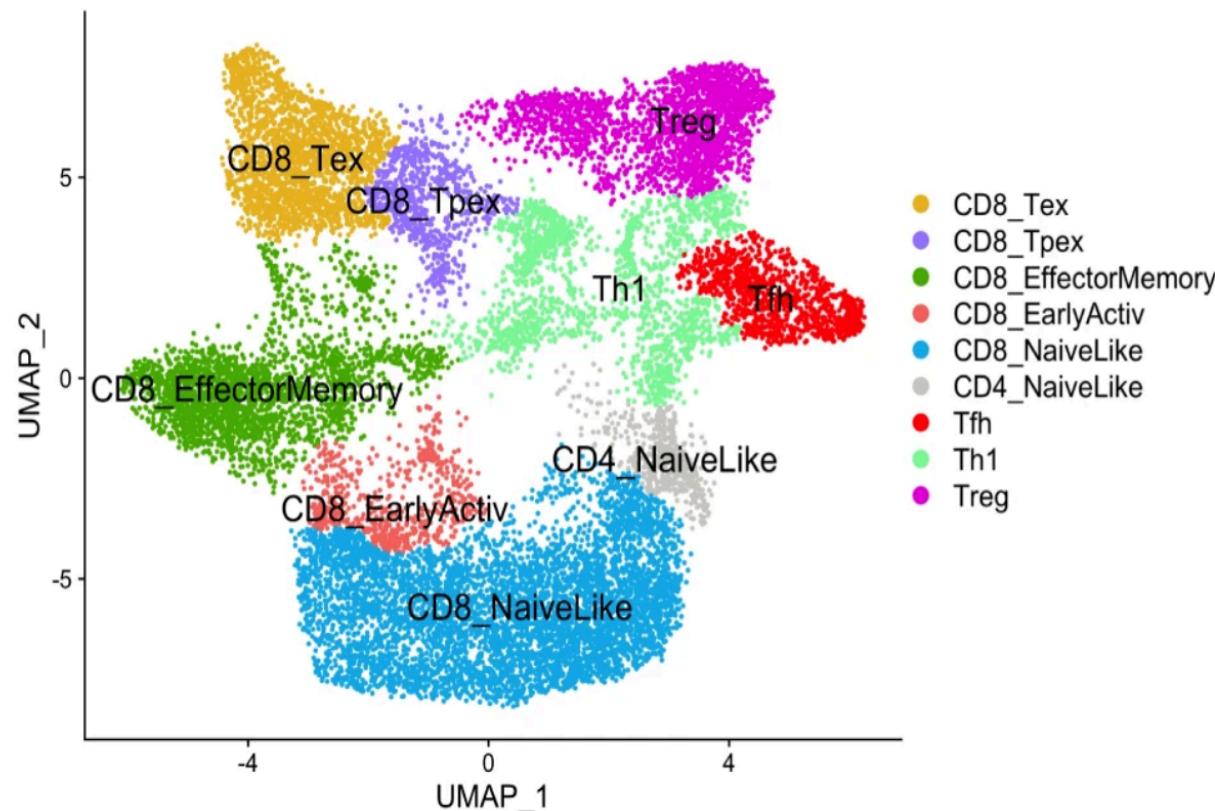
The development of Azimuth is led by the New York Genome Center Mapping Component as part of the NIH Human Biomolecular Atlas Project (HuBMAP). Six molecular reference maps are currently available, with more coming soon.

## References



<https://azimuth.hubmapconsortium.org/>

Area of development: to combine published scRNAseq datasets to create an atlas that can be used as a reference



ProjecTILs, an algorithm for reference atlas projection

Andreatta et al 2021 Nat. Comm. <https://www.nature.com/articles/s41467-021-23324-4>

# Additional links

<https://bioconductor.org/books/release/OSCA/cell-type-annotation.html#assigning-cell-labels-from-reference-data>

Dealing with multimodal single cell data:

Argelaguet et al 2021

<https://www.nature.com/articles/s41587-021-00895-7>

Review on automated cell annotation, Pasquini et al 2021

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

# Question on cell type annotation