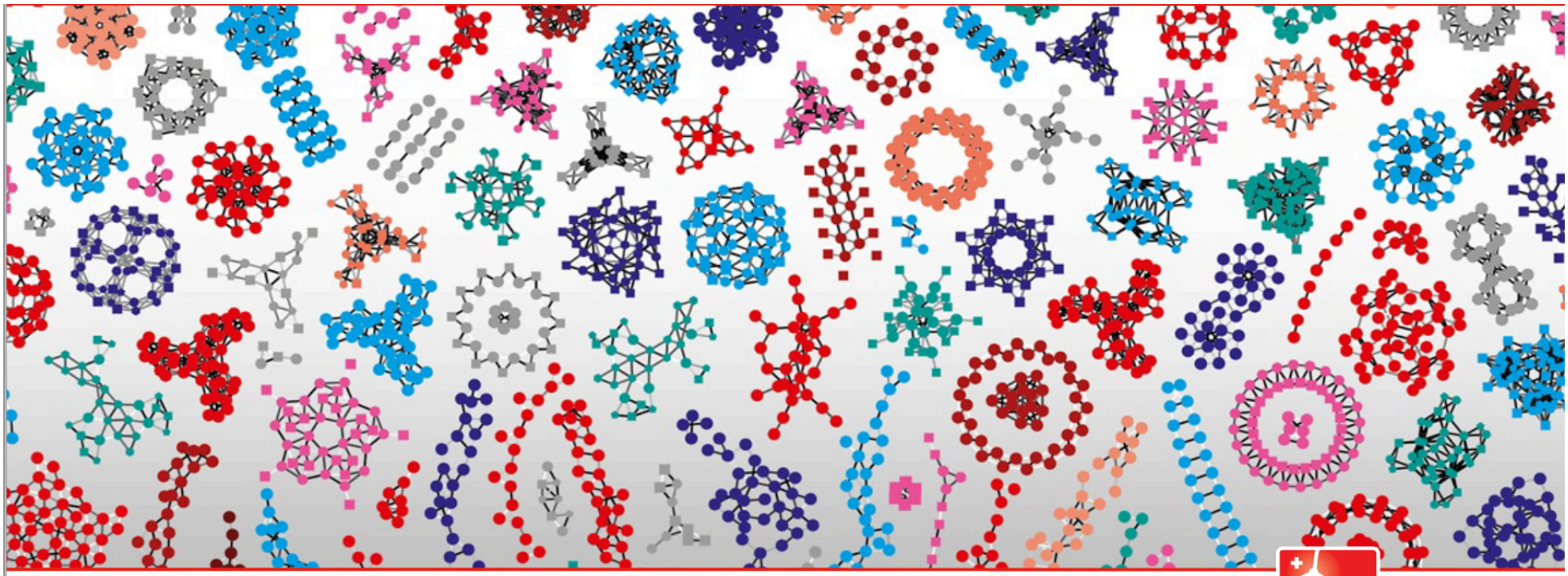


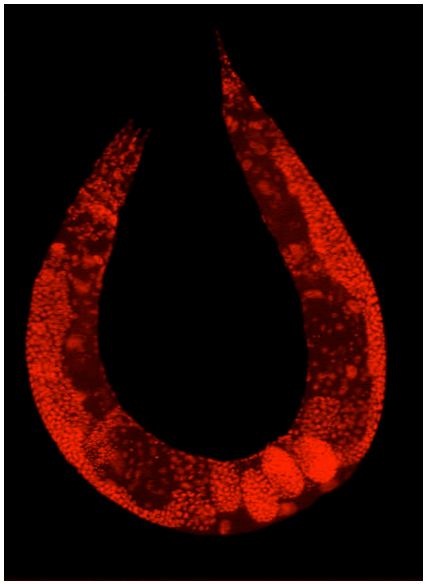
Single cell transcriptomics data analysis
Day 2

Cell type annotation

June 15th – 17th 2021

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



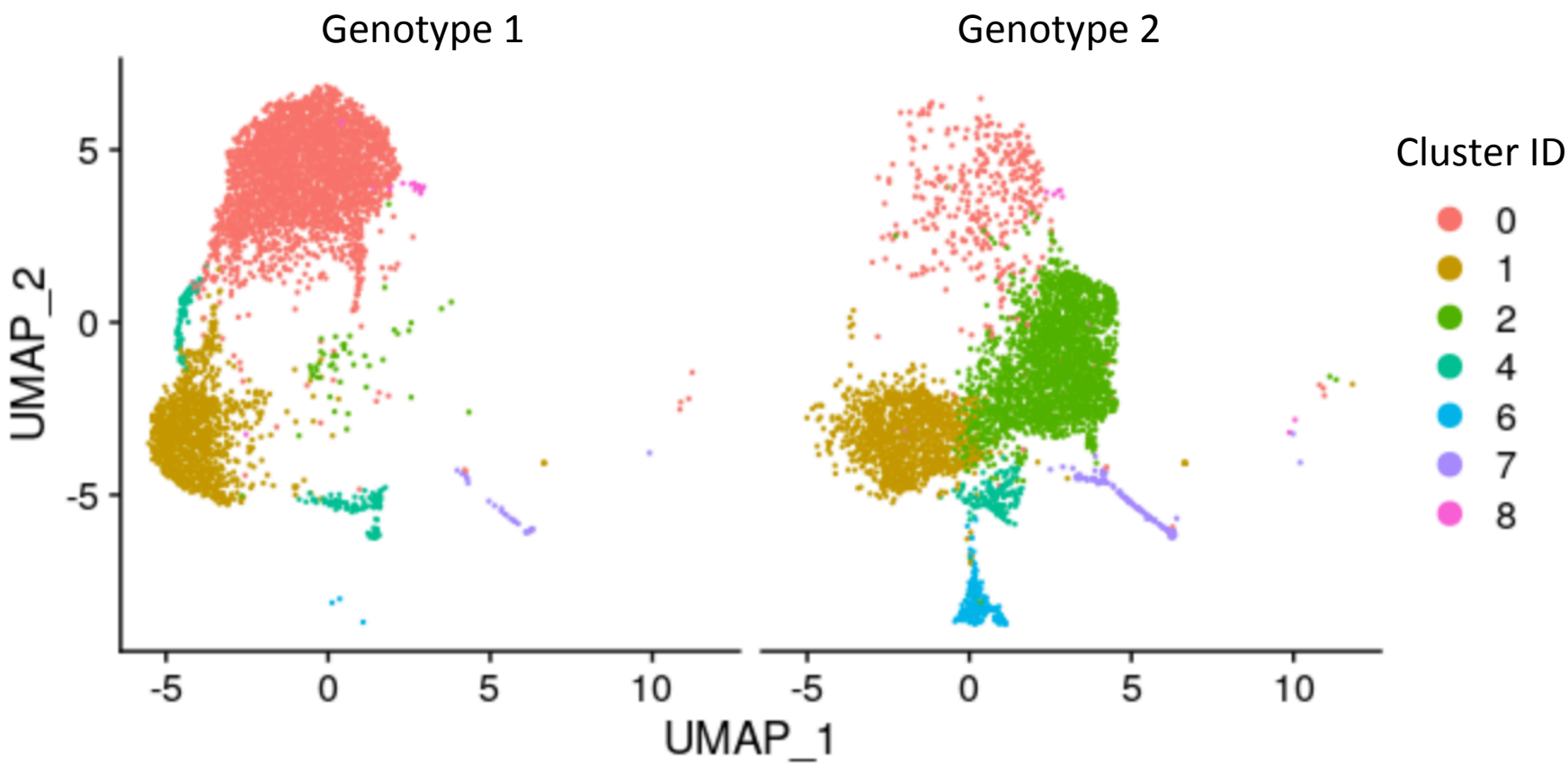
Wild-type *C. elegans* hermaphrodite stained to highlight the nuclei of all cells

Source article: (2005) The Evolution of Self-Fertile Hermaphroditism: The Fog Is Clearing. *PLoS Biol* **3**(1): e30
Creative Commons Attribution 2.5, Category:Media from PLOS journals

Why should we identify cell types?

- Sample 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?



Mouse single cells. From González-Loyola A et al, Science Advances, in press (out in July 2021)

Cell surface markers

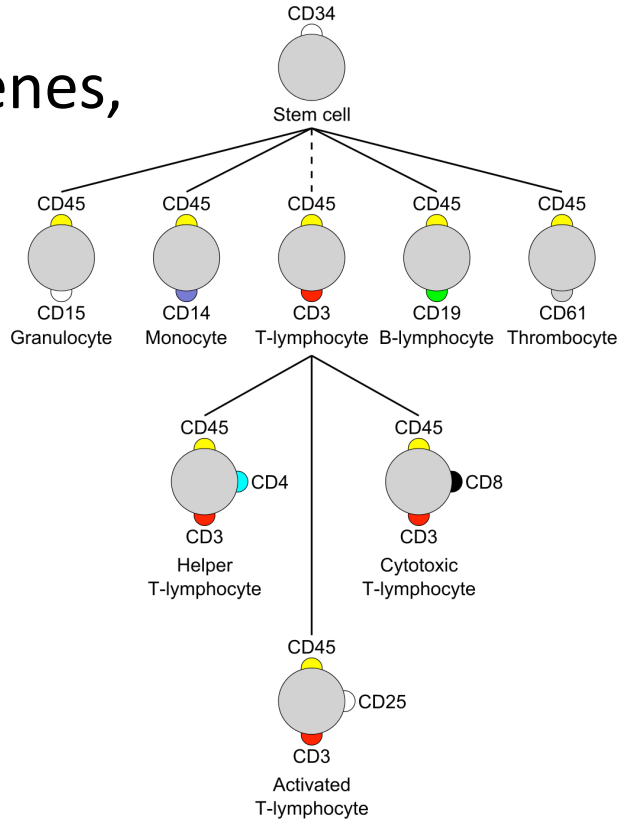
- Often considered the gold standards esp. in immunology
- mRNA of cell surface markers often lowly expressed or absent
- Use a combination of such markers genes,

  and also different genes like DE

  genes between one cluster

  compared to the other clusters

  (eg transcription factors)



By Lokal_Profil, CC BY-SA 2.5, https://commons.wikimedia.org/w/index.php?curid=8797123

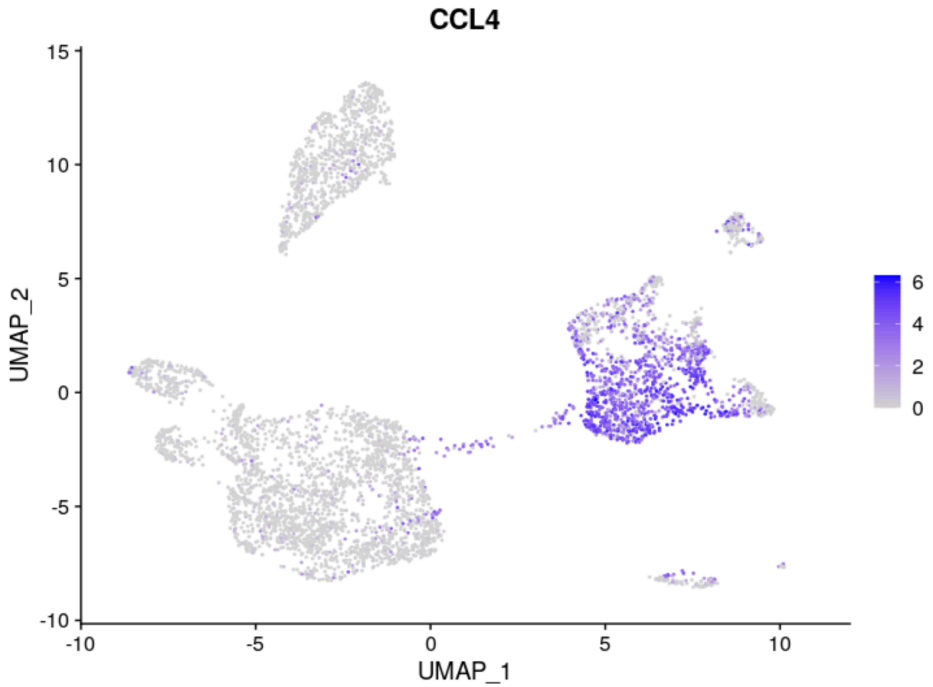
Manual *vs* automatic cell type annotation

- Manual
  - What most people do…
  - Time consuming
  - Requires expert knowledge
  - Sometimes subjective and inaccurate

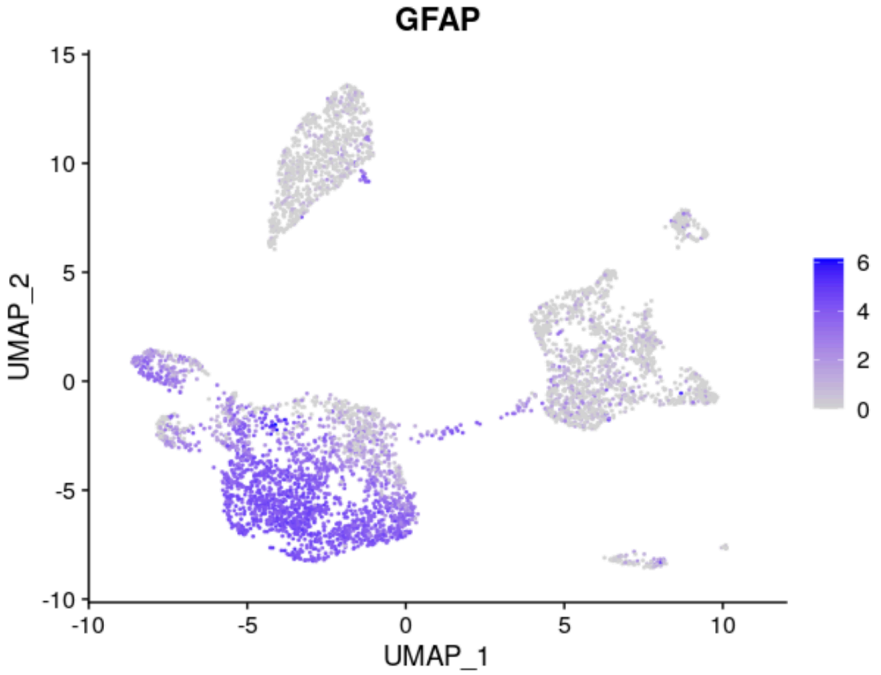
- Automatic
  - Use complete cell type-specific mRNA expression profiles based on bulk RNAseq from FACS-sorted ’pure’ populations
  - OR: Use “the reference” manually curated cells picked from scRNA-seq data sets
  - Can miss cell types if they are not included in the reference?
- Methods:
  - Assign cell type per individual cell or per cluster of cells (better per cell)
  - Mostly use “the reference” to infer per cell/cluster type by correlation

Manual annotation using known marker genes

Microglia



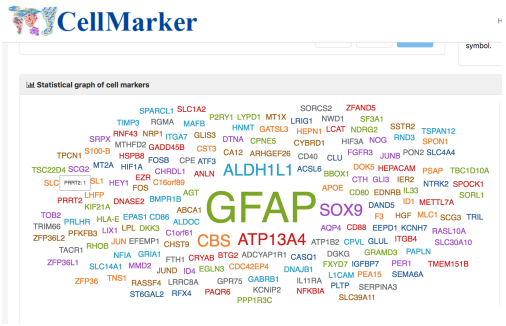
Astrocytes



Human glioblastoma multiforme cells, 10x Genomics data
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/

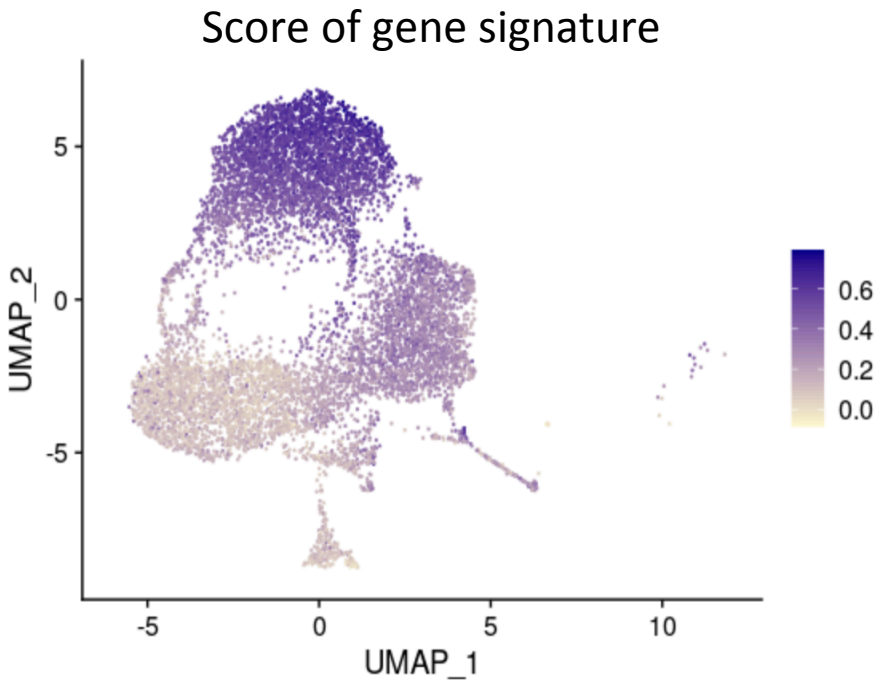
- SingleR https://github.com/dviraran/SingleR (Aran et al. 2019), access via celldex package, eg human primary cell atlas (microarrays)

- 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



Mouse single cells. From González-Loyola A et al, Science Advances, in press (out in July 2021)

SingleR

SingleR

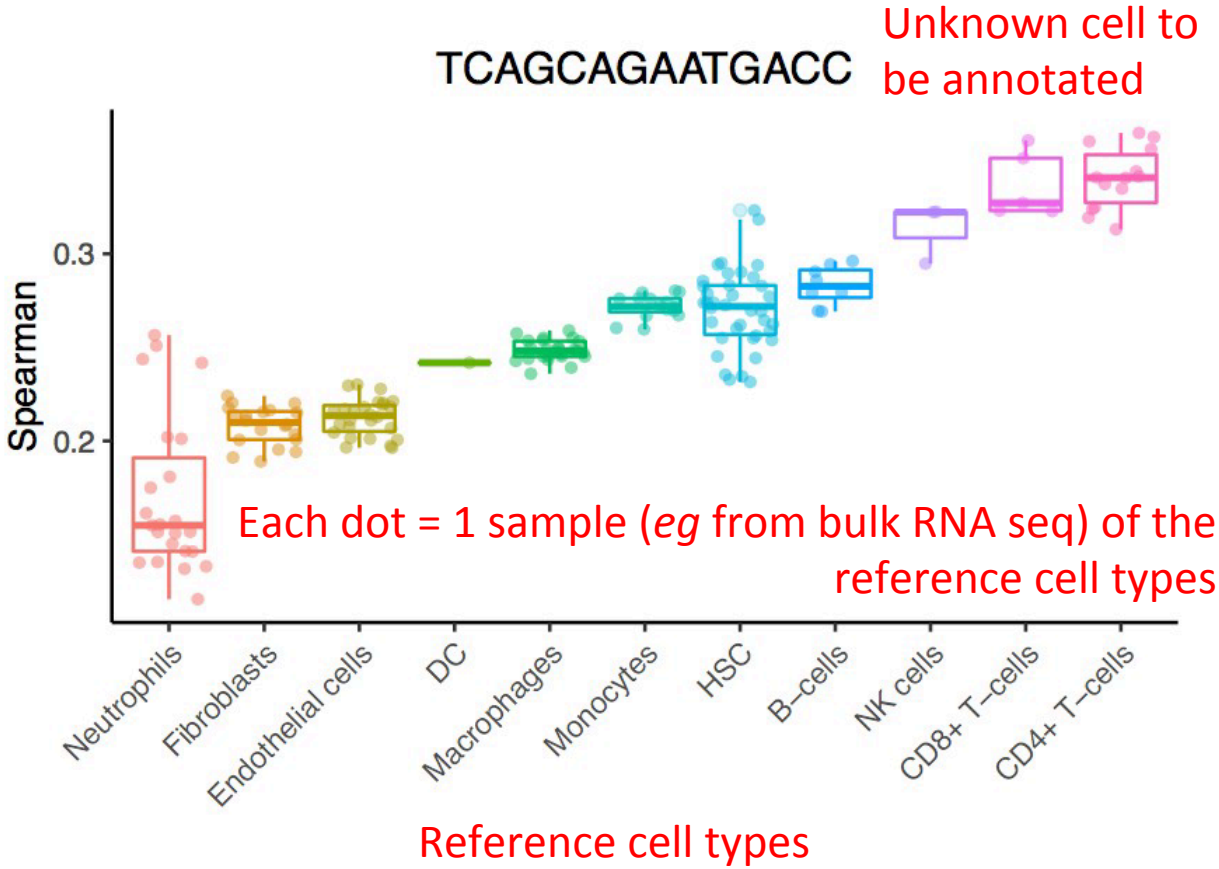
Easy access to rich reference data:

- 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, and both single cell-wise andcluster-wise

SingleR

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



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

Fine Tuning step…

Several methods 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]
scID	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]
$SVM_{rejection}$	0.19.2	Python	SVM (linear kernel)	No	Yes	[29]
kNN	0.19.2	Python	kNN ($k = 9$)	No	No	[29]

Abdelaal et al 2019
https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1795-z

Seurat, Azimuth

Cell type label transfer

Seurat 4.0.3 | Install | Get started | Vignettes | Extensions | FAQ | News | Reference | Archive

Mapping and annotating query datasets

Compiled: 2021-06-14

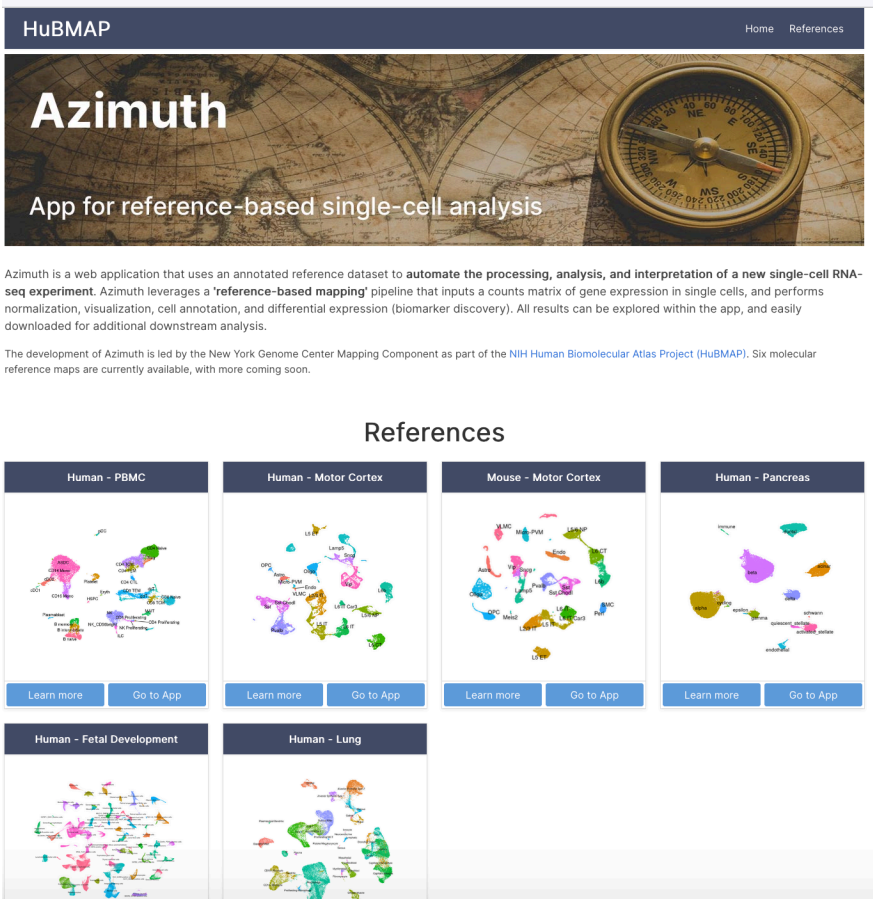
Source: `vignettes/integration_mapping.Rmd`

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.

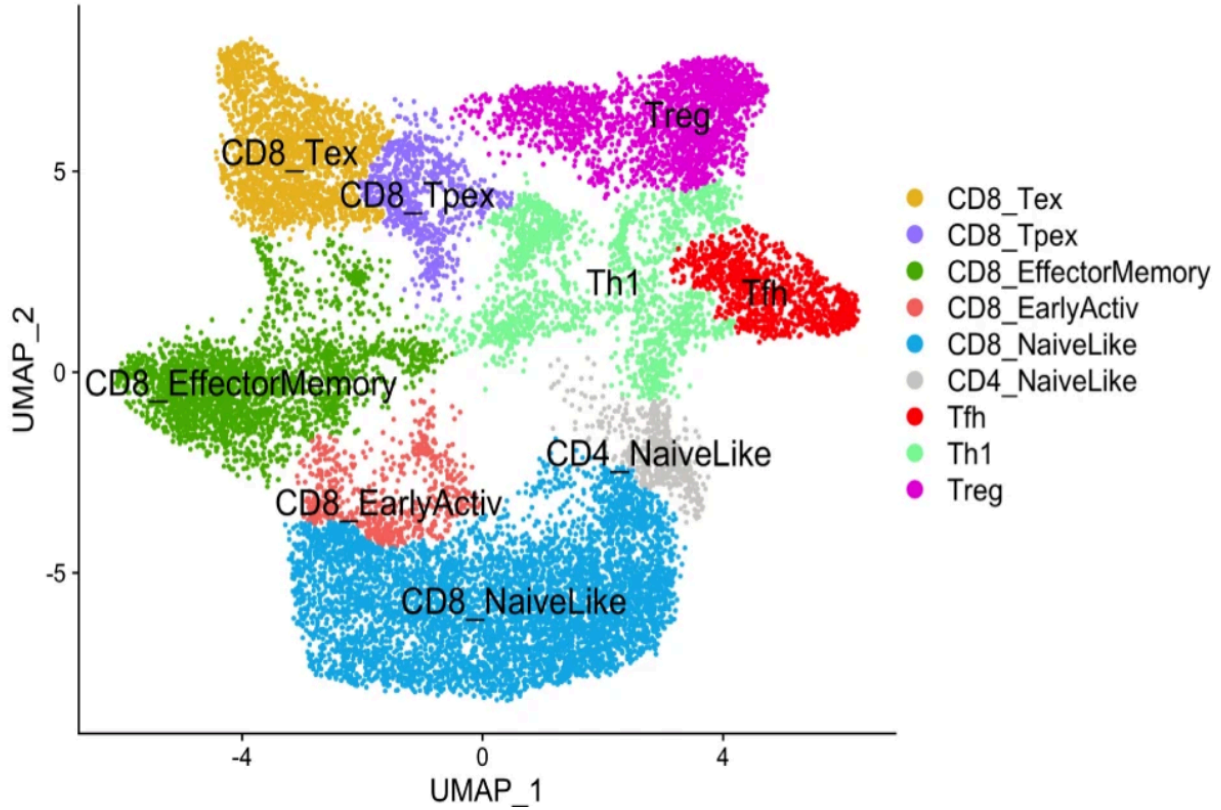
https://satijalab.org/seurat/articles/integration_mapping.html

Reference-based mapping



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