Automatic cell type annotation

相關類別

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https://reurl.cc/94ylMO

1. Overview of cell annotation workflow

Single-cell transcriptomics can profile thousands of cells in a single experiment and identify novel cell types, states and dynamics in a wide variety of tissues and organisms. Standard experimental protocols and analysis workflows have been developed to create single-cell transcriptomic maps from tissues. This tutorial focuses on how to interpret these data to identify cell types, states and other biologically relevant patterns with the objective of creating an annotated map of cells. We recommend a three-step workflow including automatic cell annotation (wherever possible), manual cell annotation and verification. Frequently encountered challenges are discussed, as well as strategies to address them. Guiding principles and specific recommendations for software tools and resources that can be used for each step are covered, and an R notebook is included to help run the recommended workflow. Basic familiarity with computer software is assumed, and basic knowledge of programming (e.g., in the R language) is recommended.

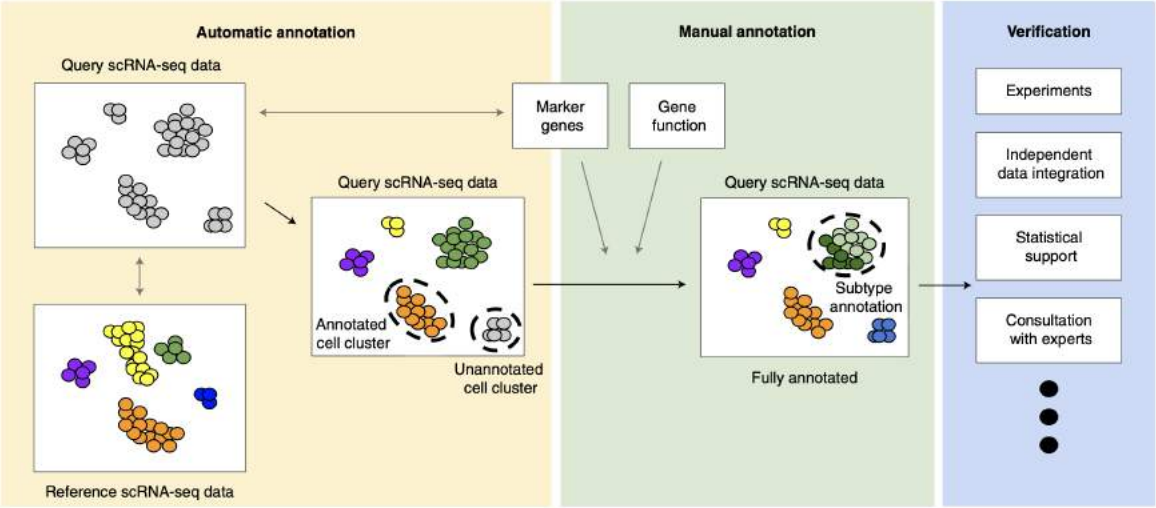

Fig. 2 | Cell annotation workflow. The recommended cell annotation process is composed of three major steps: automatic cell annotation, manual cell annotation and verification. The scRNA-seq data typically enter the workflow as a clustered gene-by-cell matrix, which is visualized using a dimensionality reduction method. An automatic cell annotation method is used to annotate cells either by comparison of the data with annotated reference data (e.g., a single-cell atlas) or using known marker genes indicative of a specific cell type. Manual annotation confirms or provides further detail for annotated cells or clusters or identifies the cell type of unlabeled clusters. Cell type can be manually inferred using a combination of marker genes, pathway analysis and differentially expressed genes with known functional information. Cell annotations are often verified using independent sources, such as new validation experiments, or comparison to complementary data, such as spatial transcriptomics data.

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▼ Comparison of the caveats and recommendations for different approaches to cell annotation

Table 1 | Comparison of the caveats and recommendations for different approaches to cell annotation

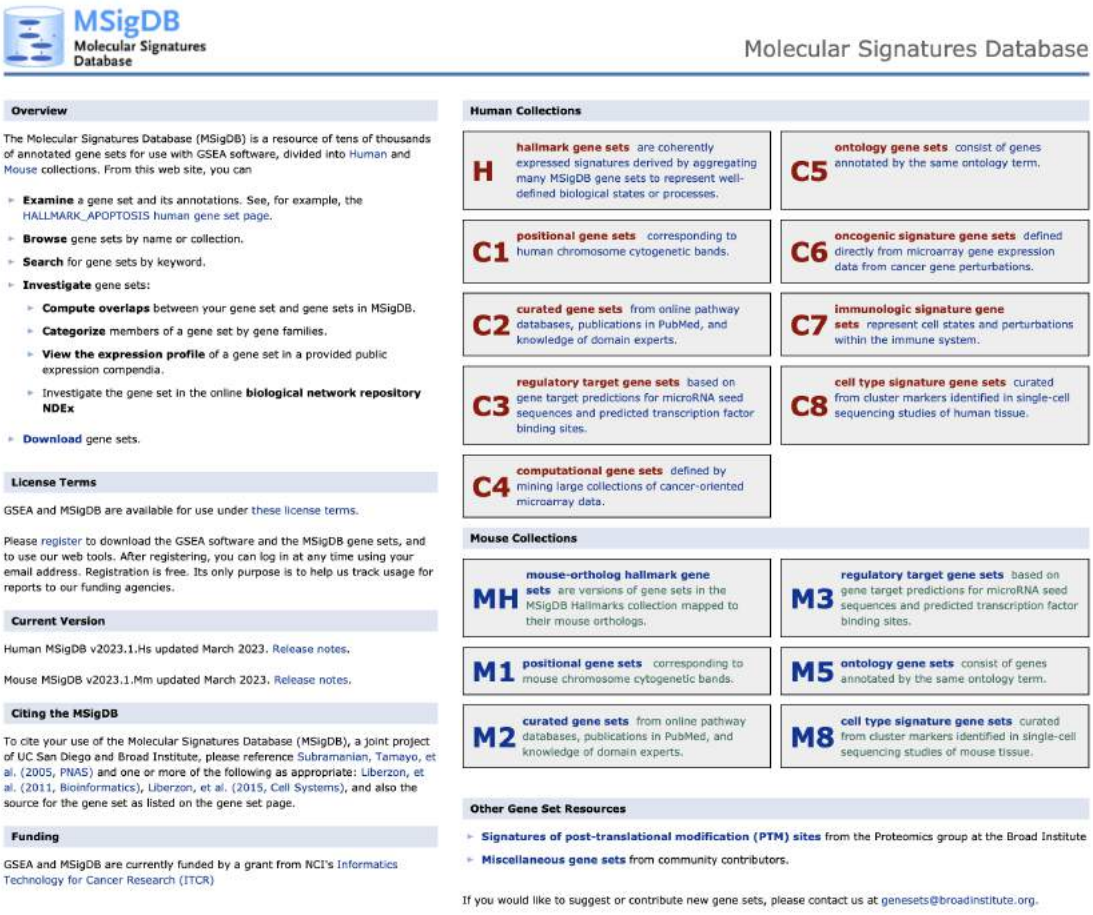
Stage of analysis	Aspect of analysis	Potential caveats	Recommendation
Automatic cell annotation	All automatic methods	Fast, but not effective for poorly characterized cells	Use manual annotation for poorly characterized cells
	Annotating clusters	May miss important differences between cells	Use automatic annotation of clusters to get a general idea of cell type and then refine labels manually. In addition, use multiple cluster-based methods and compare results
	Annotating individual cells	Ideal, but requires high reads per cell	Experiments with low reads per cell require cluster-based annotation
	Marker-based annotation methods	Marker genes not easily accessible for all cell types; may result in conflicting or absent cell labels	Requires expert knowledge to curate more extensive marker lists
	Reference-based annotation methods	Perform poorly with incomplete or poorly matched reference data, which may result in conflicting or absent cell labels	Use well-matched reference data or marker-based methods if such data are unavailable
		Often requires batch correction, which may reduce the accuracy of results	Analyze the reference data for strong biological signals. Use a good experimental protocol that will prevail over batch effects
		Mistakes in reference data get carried over to results	Analyze reference data for potential errors before using
	Comparing results from different automatic annotation methods	Results may not agree with each other	Compare confidence scores of respective labels and consider label agreement (majority rule); resolve conflicts using manual annotation
			Consider the possibility of cell subtypes, new cell types or gradients and cell states
Expert manual cell annotation	All manual methods	Slow, labor-intensive	Whenever possible, begin with automatic annotation to determine general cell labels
		Subjective	Work with an expert; consider multiple cell-type conclusions
	Marker-based annotation	Cell types not distinguishable by a single marker	Use multiple markers for each cell type
		Known markers not distinguishing cell types	Curate larger lists of markers from the literature, additional experiments or experts
		Conflicting marker gene sets between sources	Select a marker gene set that best represents the biological signal being looked for in the data (e.g., if looking for cell subtypes, use more extensive gene sets than what is used for general cell-type annotation)

2. Strategies for automatic cell annotation

2-1 Marker-based annotation approach

- Characteristic expression of **known marker genes**
- Known relationships between **marker genes** and **cell types** from **databases**

▼ **MsigDB** (https://www.gsea-msigdb.org/gsea/msigdb)



MSigDB
Molecular Signatures Database

Molecular Signatures Database

Overview

The Molecular Signatures Database (MSigDB) is a resource of tens of thousands of annotated gene sets for use with GSEA software, divided into Human and Mouse collections. From this web site, you can

- **Examine** a gene set and its annotations. See, for example, the HALLMARK_APOPTOSIS human gene set page.
- **Browse** gene sets by name or collection.
- **Search** for gene sets by keyword.
- **Investigate** gene sets:
  - **Compute overlaps** between your gene set and gene sets in MSigDB.
  - **Categorize** members of a gene set by gene families.
  - **View the expression profile** of a gene set in a provided public expression compendia.
  - Investigate the gene set in the online **biological network repository NDEx**
- **Download** gene sets.

License Terms

GSEA and MSigDB are available for use under these license terms.

Please register to download the GSEA software and the MSigDB gene sets, and to use our web tools. After registering, you can log in at any time using your email address. Registration is free. Its only purpose is to help us track usage for reports to our funding agencies.

Current Version

Human MSigDB v2023.1.Hs updated March 2023. Release notes.

Mouse MSigDB v2023.1.Mm updated March 2023. Release notes.

Citing the MSigDB

To cite your use of the Molecular Signatures Database (MSigDB), a joint project of UC San Diego and Broad Institute, please reference Subramanian, Tamayo, et al. (2005, PNAS) and one or more of the following as appropriate: Liberzon, et al. (2011, Bioinformatics), Liberzon, et al. (2015, Cell Systems), and also the source for the gene set as listed on the gene set page.

Funding

GSEA and MSigDB are currently funded by a grant from NCI's Informatics Technology for Cancer Research (ITCR)

Human Collections

- **H** — **hallmark gene sets** are coherently expressed signatures derived by aggregating many MSigDB gene sets to represent well-defined biological states or processes.
- **C5** — **ontology gene sets** consist of genes annotated by the same ontology term.
- **C1** — **positional gene sets** corresponding to human chromosome cytogenetic bands.
- **C6** — **oncogenic signature gene sets** defined directly from microarray gene expression data from cancer gene perturbations.
- **C2** — **curated gene sets** from online pathway databases, publications in PubMed, and knowledge of domain experts.
- **C7** — **immunologic signature gene sets** represent cell states and perturbations within the immune system.
- **C3** — **regulatory target gene sets** based on gene target predictions for microRNA seed sequences and predicted transcription factor binding sites.
- **C8** — **cell type signature gene sets** curated from cluster markers identified in single-cell sequencing studies of human tissue.
- **C4** — **computational gene sets** defined by mining large collections of cancer-oriented microarray data.

Mouse Collections

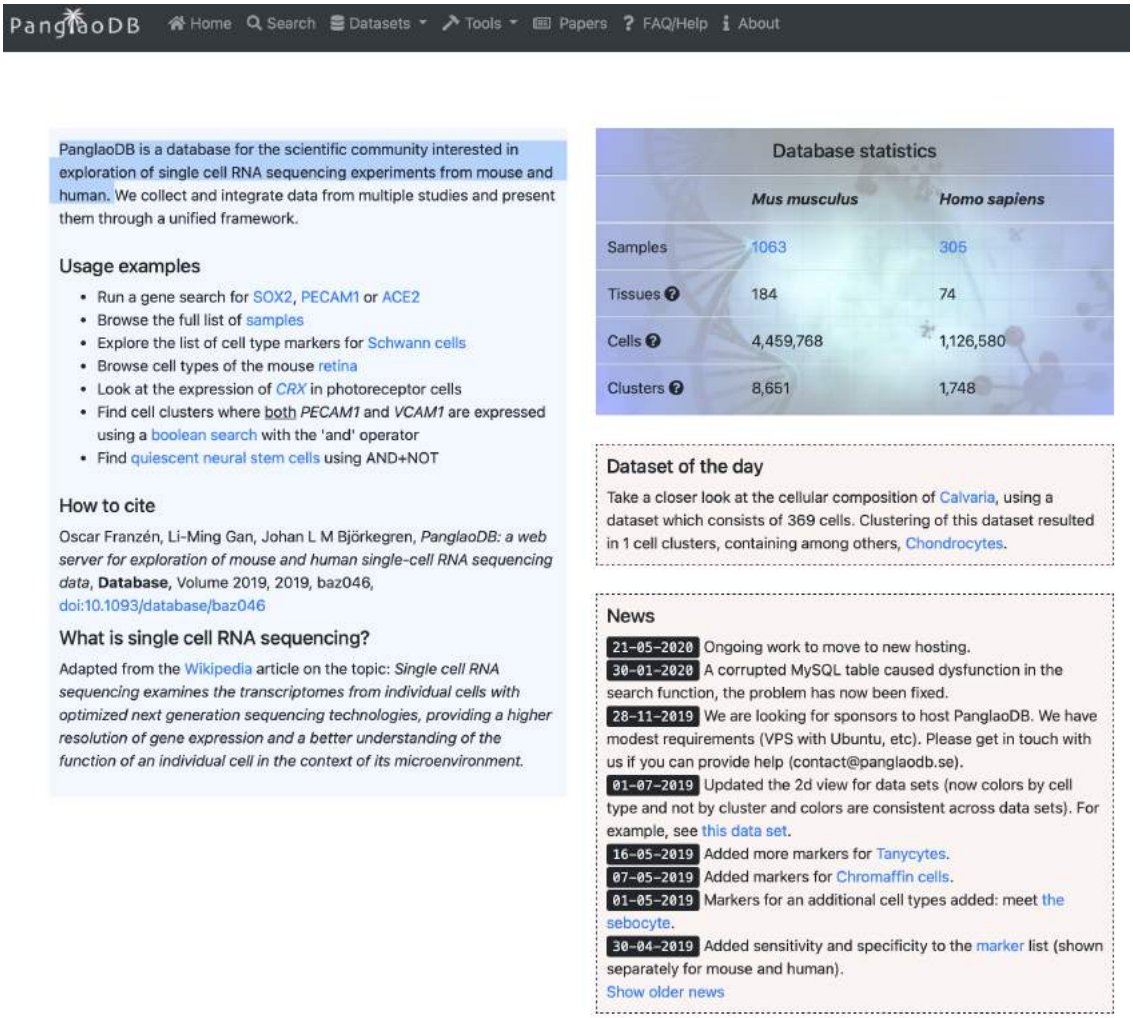
- **MH** — **mouse-ortholog hallmark gene sets** are versions of gene sets in the MSigDB Hallmarks collection mapped to their mouse orthologs.
- **M3** — **regulatory target gene sets** based on gene target predictions for microRNA seed sequences and predicted transcription factor binding sites.
- **M1** — **positional gene sets** corresponding to mouse chromosome cytogenetic bands.
- **M5** — **ontology gene sets** consist of genes annotated by the same ontology term.
- **M2** — **curated gene sets** from online pathway databases, publications in PubMed, and knowledge of domain experts.
- **M8** — **cell type signature gene sets** curated from cluster markers identified in single-cell sequencing studies of mouse tissue.

Other Gene Set Resources

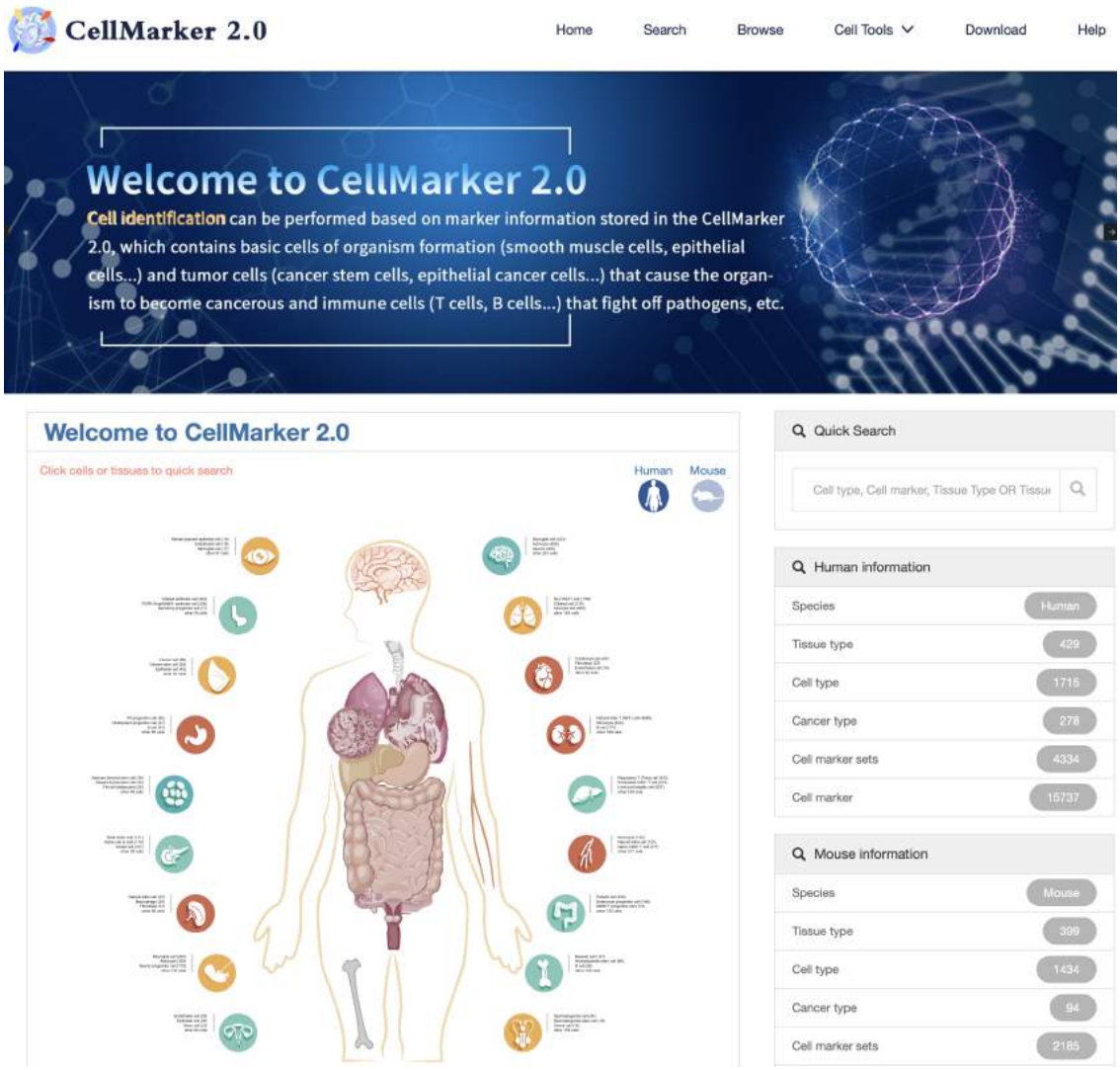
- **Signatures of post-translational modification (PTM) sites** from the Proteomics group at the Broad Institute
- **Miscellaneous gene sets** from community contributors.

If you would like to suggest or contribute new gene sets, please contact us at genesets@broadinstitute.org.

▼ **PanglaoDB** (https://panglaodb.se)



▼ **CellMarker** (http://117.50.127.228)



▼ **Literatures**

AUCell[103]	Marker based	R	Single cells	Area under the curve to estimate marker gene set enrichment	Yes	Because of low detection rates at the level of single cells, it requires many markers for every cell type
SCINA[34]	Marker based	R	Single cells	Expectation maximization, Gaussian mixture model	(Optional)	Simultaneously clusters and annotates cells; robust to the inclusion of incorrect marker genes
GSEA/GSVA[36,104]	Marker based	R/Java	Clusters of cells	Enrichment test	Yes	Marker gene lists must be reformatted in GMT format. Markers must all be differentially expressed in the same direction in the cluster

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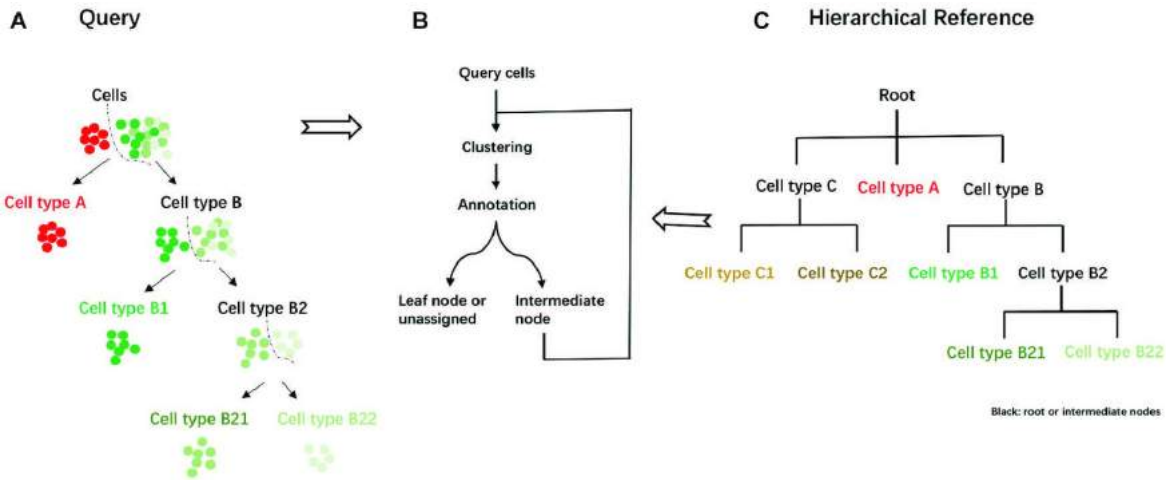
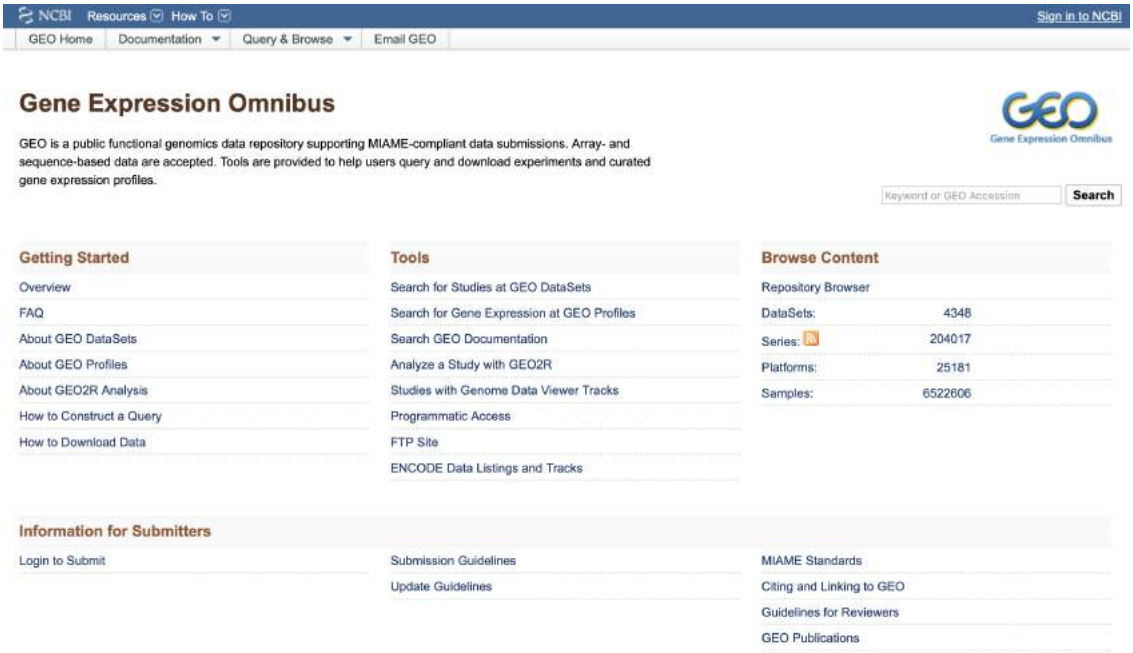
▼ **scMRMA: single cell multiresolution marker-based annotation** *Nucleic Acids Research*, Volume 50, Issue 2, 25 January 2022, Page e7,



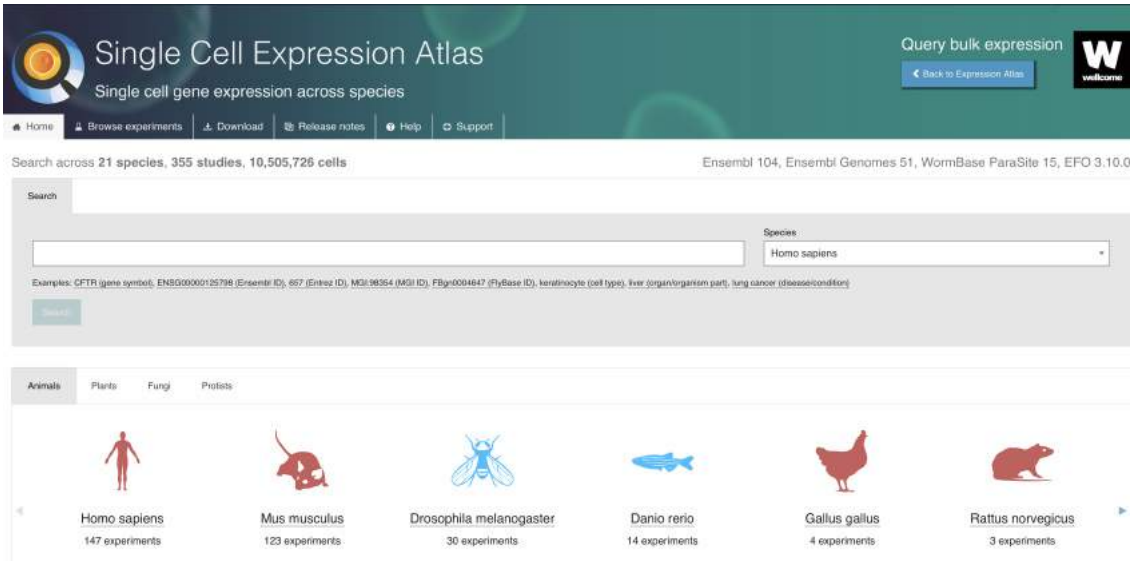
2-2 Reference-based annotation approach

- transfer labels from **reference cell or cluster (well annotated scRNA-seq data)**
- Reference single-cell data are obtained from

▼ Gene Expression Omnibus (GEO https://www.ncbi.nlm.nih.gov/geo/)



▼ Single Cell Expression Atlas (https://www.ebi.ac.uk/gxa/sc/home)



▼ Cell atlas projects

1. Human Cell Atlas (HCA) - The HCA aims to create comprehensive reference maps of all human cells, including their molecular profiles, for a better understanding of human health and disease.

   Website: https://www.humancellatlas.org/

2. Mouse Cell Atlas (MCA) - The MCA project focuses on generating a comprehensive cell atlas of the mouse, providing a valuable resource for understanding mouse development, physiology, and disease models.
   Website: https://www.mousecellatlas.org/

3. Human Protein Atlas (HPA) - The HPA aims to map the location of all human proteins in cells, tissues, and organs using various omics technologies, enabling researchers to explore protein expression and localization patterns.
   Website: https://www.proteinatlas.org/

4. Tabula Muris - The Tabula Muris project focuses on creating a single-cell transcriptomic atlas of different organs and tissues in the mouse, providing insights into cellular diversity and function.
   Website: https://tabula-muris.ds.czbiohub.org/

5. Fly Cell Atlas - The Fly Cell Atlas project aims to comprehensively map and characterize cell types in the fruit fly Drosophila melanogaster, enabling insights into fly development and physiology.
   Website: https://www.flycellatlas.org/

singleCell Net[42]	Reference based	R	Single cells	Relative-expression gene pairs + random forest	Yes, but rarely does so even when it should[33]	10–100× slower than other methods; high accuracy
scmap-cluster[41]	Reference based	R	Single cells	Consistent correlations	Yes	Fastest method available; balances false-positives and false-negatives; includes web interface for use with a large pre-built reference or custom reference set
scmap-cell[41]	Reference based	R	Single cells	Approximate nearest neighbors	Yes	Assigns individual cells to nearest neighbor cells in reference; allows mapping of cell trajectories; fast and scalable
singleR[43]	Reference based	R	Single cells	Hierarchical clustering and Spearman correlations	No	Includes a large marker reference; does not scale to data sets of ≥10,000 cells; includes web interface with marker database
Scikit-learn[102]	Reference based	Python	Multiple possible	k-nearest neighbors, support vector machine, random forest, nearest mean classifier and linear discriminant analysis	(Optional)	Expertise required for correct design and appropriate training of classifier while avoiding overtraining

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2-3 Strengths & pitfalls of marker-based and reference-based annotation approaches

Annotation Method	Strengths	Pitfalls

Marker-based	Fast and efficient for well-characterized cell types	May not be effective for poorly characterized or rare cell types
	Can be used to identify specific cell types based on known markers	May result in conflicting or absent cell labels if marker genes are not easily accessible for all cell types
		Requires expert knowledge to curate more extensive marker lists
Reference-based	Can be used to identify novel cell types or subtypes based on similarity to reference data	Requires high-quality and relevant annotated reference data
	Can be more accurate than marker-based methods for well-matched reference data	May perform poorly with incomplete or poorly matched reference data, which may result in conflicting or absent cell labels
	Can be used to integrate data from multiple sources	Often requires batch correction, which may reduce the accuracy of results

▼ **ChatPDF**

s41596-021-00534-0.pdf

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https://www.chatpdf.com/

3. Reference-based annotation of scRNA-Seq (SingleR)

3-1 Cell type reference datasets (celldex package)

(2019) “Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage.”

Nat. Immunol., **20**, 163-172. doi:10.1038/s41590-018-0276-y.

▼ **Human (5)**

BlueprintEncodeData

Blueprint (Martens and Stunnenberg 2013) and Encode (The ENCODE Project Consortium 2012)

DatabaseImmuneCellExpressionData

The Database for Immune Cell Expression(/eQTLs/Epigenomics)(Schmiedel et al. 2018)

HumanPrimaryCellAtlasData

The Human Primary Cell Atlas (Mabbott et al. 2013)

MonacoImmuneData

Monaco Immune Cell Data - GSE107011 (Monaco et al. 2019)

NovershternHematopoieticData

Novershtern Hematopoietic Cell Data - GSE24759

▼ **Mouse (2)**

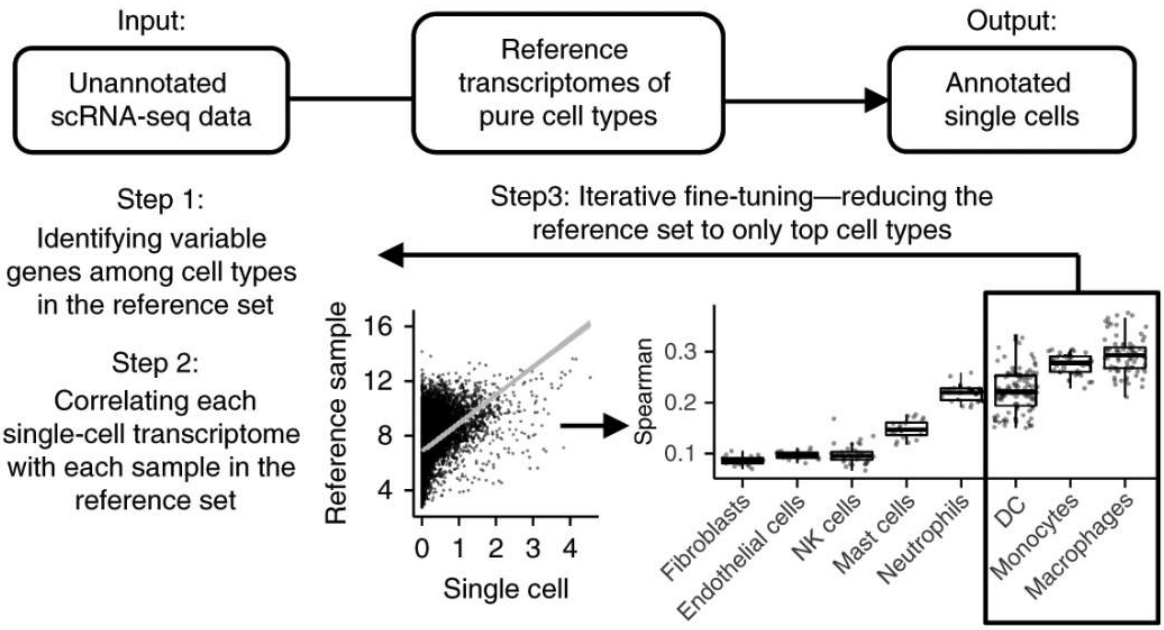
ImmGenData

the murine ImmGen (Heng et al. 2008)

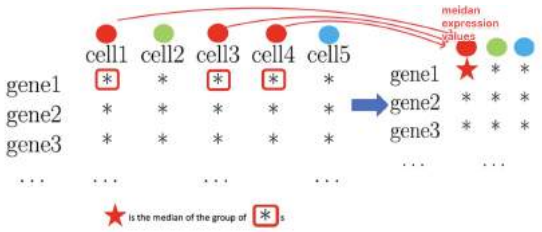
MouseRNAseqData

a collection of mouse data sets downloaded from GEO (Benayoun et al. 2019)

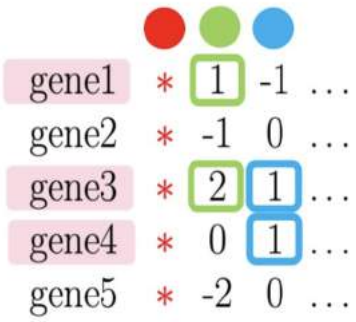
3-2 Schematic of SingleR



▼ Step1: Identifying variable genes



For each gene, the **median** expression values were obtained by grouping them according to cell type.

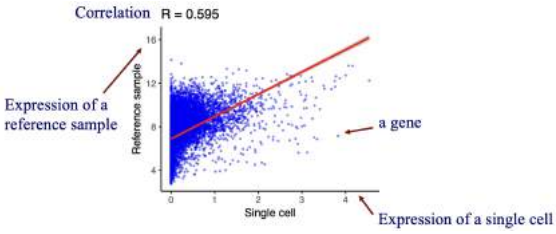


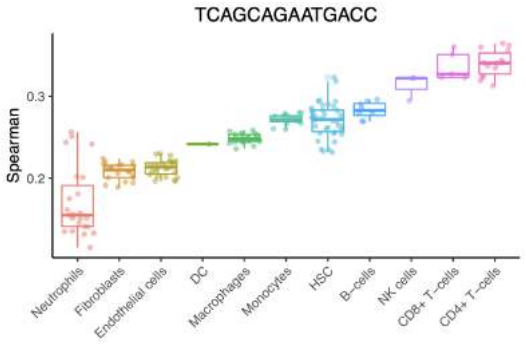
- Differential expression between each other cell type and the **'red'** cell type was

  calculated and all genes with positive differential expression values were

  selected
- The top *N* genes that showed the most difference in expression were chosen for the "red" cell type as **variable genes**.

▼ Step2: Correlation analysis

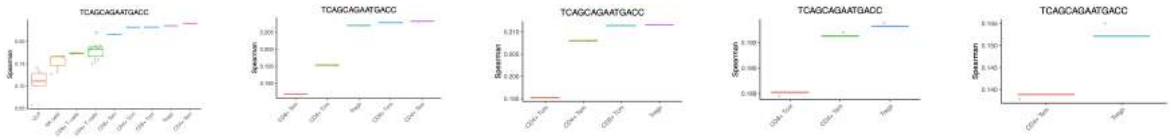




▼ Step3: Iterative fine-tuning (variable genes)

In this step *SingleR* reruns the correlation analysis, but only for the top cell types from step 2.

The analysis is performed only on **variable genes** between these cell types.

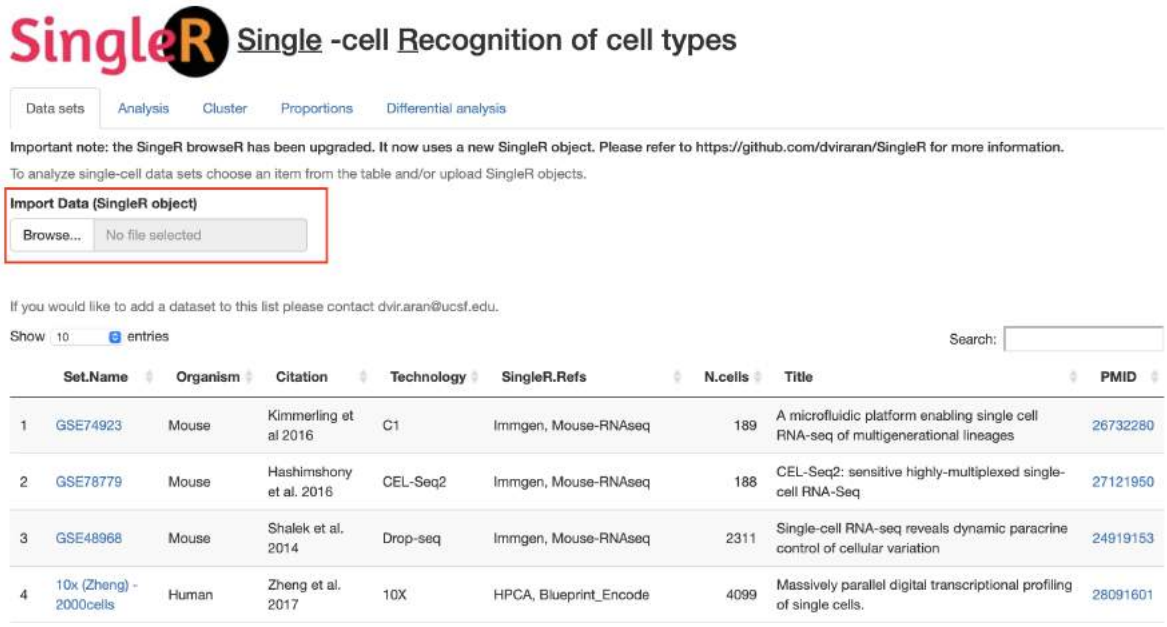


3-3 SingleR browser

singler - Default

https://comphealth.ucsf.edu/app/singler

▼ *SingleR browser*



	Set.Name	Organism	Citation	Technology	SingleR.Refs	N.cells	Title	PMID
1	GSE74923	Mouse	Kimmerling et al 2016	C1	Immgen, Mouse-RNAseq	189	A microfluidic platform enabling single cell RNA-seq of multigenerational lineages	26732280
2	GSE78779	Mouse	Hashimshony et al. 2016	CEL-Seq2	Immgen, Mouse-RNAseq	188	CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq	27121950
3	GSE48968	Mouse	Shalek et al. 2014	Drop-seq	Immgen, Mouse-RNAseq	2311	Single-cell RNA-seq reveals dynamic paracrine control of cellular variation	24919153
4	10x (Zheng) - 2000cells	Human	Zheng et al. 2017	10X	HPCA, Blueprint_Encode	4099	Massively parallel digital transcriptional profiling of single cells.	28091601

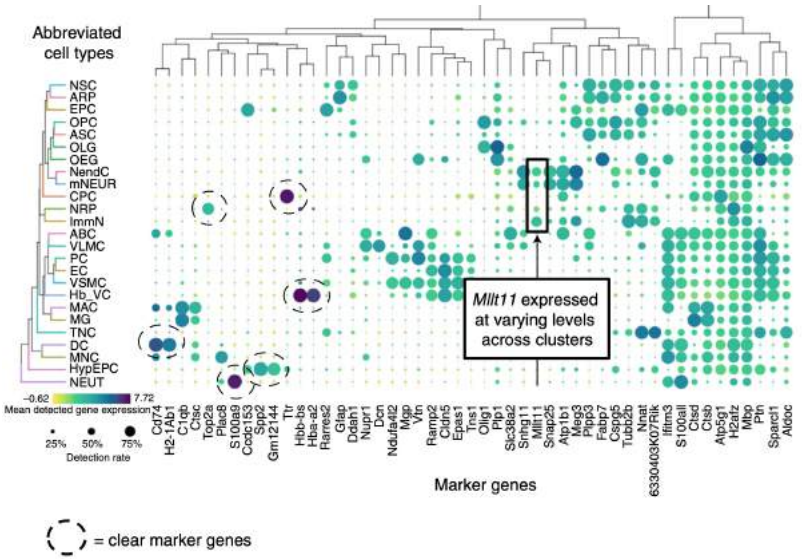
```
library(SingleR)

# Simplest use is running the wrapper function that creates both a SingleR and Seurat object:
```

```
# counts.file maybe a tab delimited text file, 10X directory or a matrix. annot is a tab delimited
# text file or a data.frame with the original identities. normalize.gene.length should be true if
# the data comes from a full-length platform. min.genes, min.cells, npca and regress.out are passed
# to Seurat to create a Seurat object object:
singler = CreateSinglerSeuratObject(counts.file, annot, project.name,
  min.genes = 500, technology, species = "Human" (or "Mouse"), citation,
  normalize.gene.length = F, min.cells = 2, npca = 10
  regress.out = "nUMI", reduce.seurat.object = T)

# The object can then be saved and uploaded to the SingleR web-app for further analysis and visualization or using functions avail
save(singler,file=paste0(project.name,'.RData')
```

4. Marker-based annotation of scRNA-Seq



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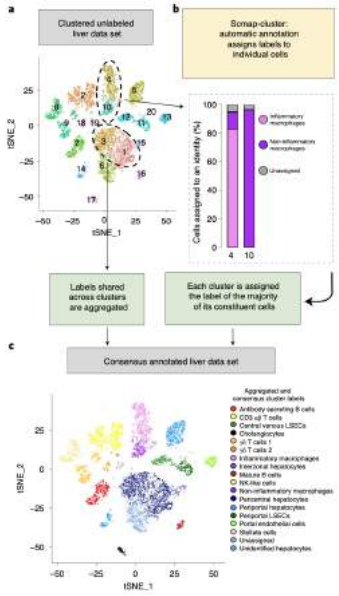
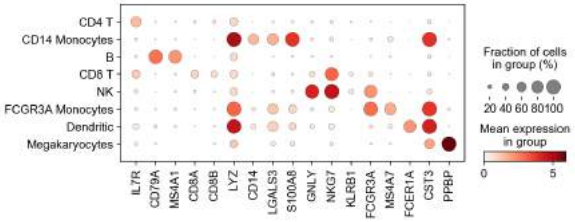
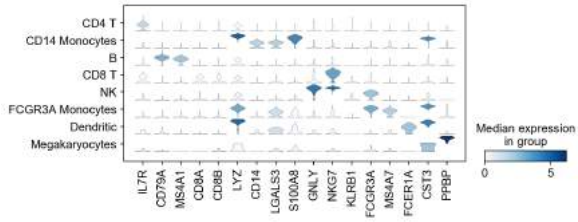


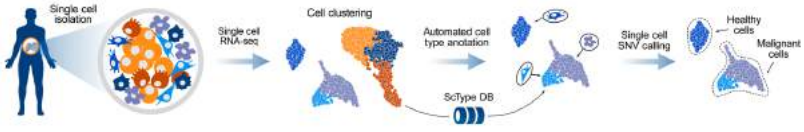


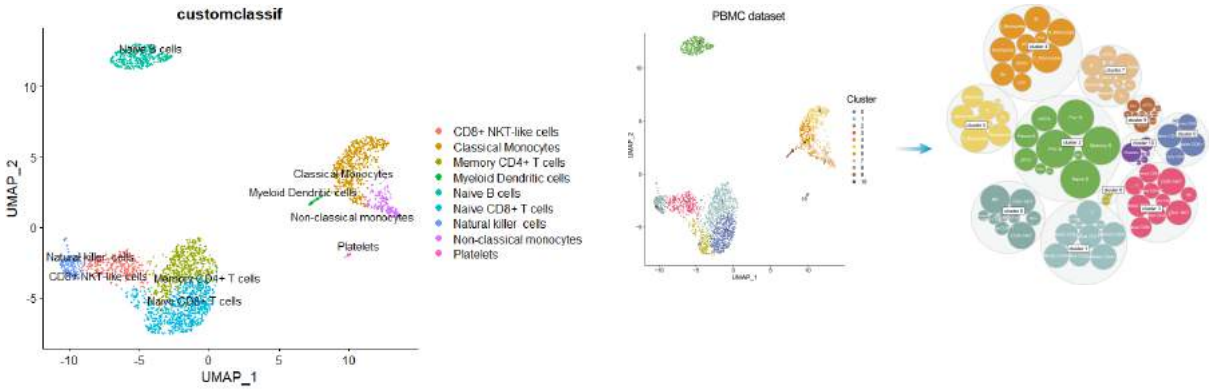
Genome Biol. 2018 Feb 6;19(1):15



Genome Biol. 2018 Feb 6;19(1):15

4-1 ScType





s41467-022-28803-w.pdf

▼ **ScType database**

- CellMarker database
  - 13,605 cell markers for **467 cell types** in 158 human tissues/sub-tissues
  - 9,148 cell makers for **389 cell types** in 81 mouse tissues/sub-tissues
- PanglaoDB
  - 6,631 gene markers mapping to **155 cell type**
- Literature search
  - 37 negative markers
- User custom sets of positive and negative markers
  - domain knowledge
  - emerging studies

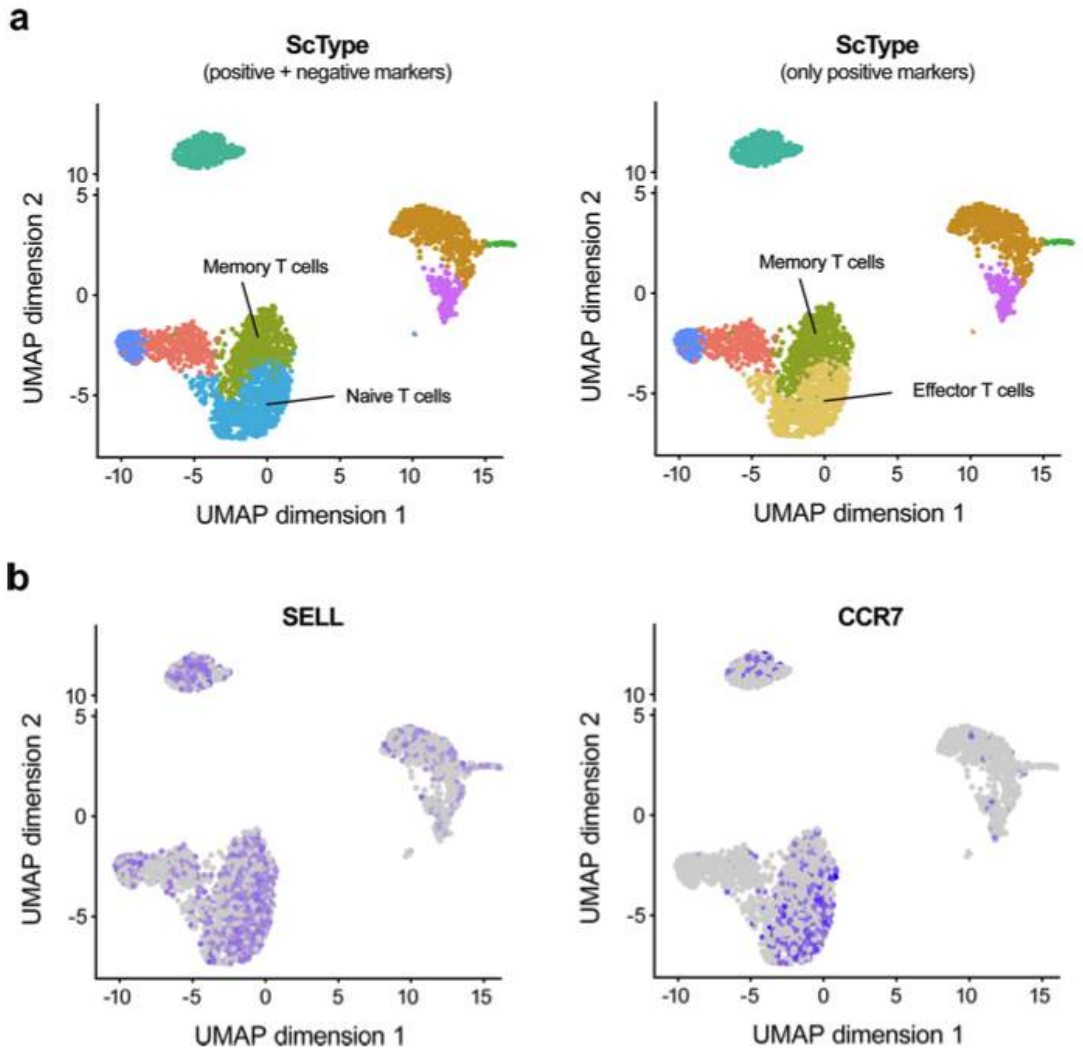
The widely applicable method is deployed both as **an interactive web-tool (https://sctype.app)**, and as **an open-source R-package (**https://github.com/IanevskiAleksandr/sc-type**)**.

sc-type

https://sctype.app/database.php

> ScType utilizes both **positive** and **negative markers** for the cell type annotation

Naïve and memory T cells express **CCR7** and **SELL genes** for lymph node migration, while effector T cells do not.



Nat Commun. 2022 Mar 10;13(1):1246.

4-2 scCATCH

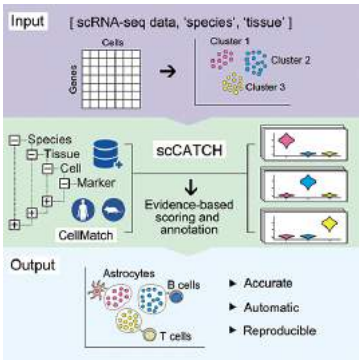
Accurate cell type identification is crucial for single-cell RNA sequencing studies, but current manual annotation methods can be time-consuming and subjective. The single cell Cluster-based Annotation Toolkit for Cellular Heterogeneity (scCATCH) offers a solution by automating the identification of cluster marker genes and annotation based on evidence-based scores and **a tissue-specific cell taxonomy reference database (CellMatch)**.

CellMatch includes a panel of **353 cell types** and **related 686 subtypes** associated with **184 tissue types**, and **2,096 references of human and mouse**.

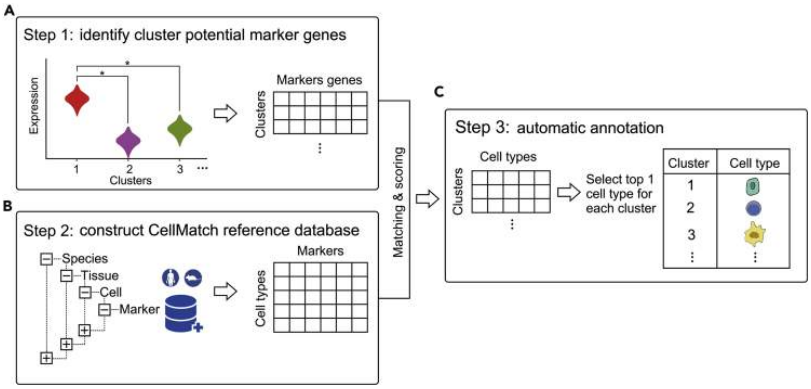
▼ CellMatch database

	species	tissue	cancer	condition	subtype1	subtype2	s
1	Human	Kidney	Normal	Normal cell	*NA*	*NA*	N
2	Human	Liver	Normal	Normal cell	*NA*	*NA*	N
3	Human	Endometrium	Normal	Normal cell	*NA*	*NA*	N
4	Human	Germ	Normal	Normal cell	Primordial	*NA*	N
5	Human	Corneal epithelium	Normal	Normal cell	*NA*	*NA*	N
6	Human	Placenta	Normal	Normal cell	*NA*	*NA*	N
7	Human	Periosteum	Normal	Normal cell	Periosteum-Derived	*NA*	N

	species	tissue	cancer	condition	subtype1	subtype2	s
8	Human	Periosteum	Normal	Normal cell	Periosteum-Derived	*NA*	*N*
9	Human	Periosteum	Normal	Normal cell	Periosteum-Derived	*NA*	*N*
10	Human	Periosteum	Normal	Normal cell	Periosteum-Derived	*NA*	*N*
11	Human	Amniotic membrane	Normal	Normal cell	Amnion	*NA*	*N*
12	Human	Amniotic membrane	Normal	Normal cell	Amnion	*NA*	*N*
13	Human	Primitive streak	Normal	Normal cell	Primitive	*NA*	*N*
14	Human	Primitive streak	Normal	Normal cell	Primitive	*NA*	*N*
15	Human	Adipose tissue	Normal	Normal cell	*NA*	*NA*	*N*
16	Human	Scalp	Normal	Normal cell	Bulge	*NA*	*N*
17	Human	Heart	Normal	Normal cell	*NA*	*NA*	*N*
18	Human	Liver	Normal	Normal cell	*NA*	*NA*	*N*
19	Human	Liver	Normal	Normal cell	*NA*	*NA*	*N*



iScience. 2020 Mar 27;23(3):100882




```
obj <- findmarkergene(object = obj,
                      species = "Human",
                      marker = cellmatch,
                      tissue = c("Blood", "Peripheral blood", "Plasma", "Serum", "Umbilical cord blood"))
```

```
obj <- findmarkergene(object = obj,
                      species = "Human",
                      marker = cellmatch,
                      tissue = c("Blood", "Peripheral blood", "Serum", "Colon", "Colorectum", "Intestine")),
                      cancer = c("Colon Cancer", "Colorectal Cancer"))
```

Allow users to select different combination of tissues or cancers for annotation

```
# Example
cellmatch_new <- cellmatch[cellmatch$species == "Mouse" & cellmatch$tissue %in% c("Kidney", "Liver", "Lung", "Brain"), ]
obj <- findmarkergene(object = obj, if_use_custom_marker = TRUE, marker = cellmatch_new)
obj <- findcelltype(obj)

# Example
cellmatch_new <- cellmatch[cellmatch$species == "Mouse" & cellmatch$cancer %in% c("Lung Cancer", "Lymph node", "Renal Cell Carcinoma",
obj <- findmarkergene(object = obj, if_use_custom_marker = TRUE, marker = cellmatch_new)
obj <- findcelltype(obj)

# Example
cellmatch_new <- cellmatch[cellmatch$species == "Mouse", ]
cellmatch_new <- cellmatch[cellmatch$cancer %in% c("Lung Cancer", "Lymph node", "Renal Cell Carcinoma", "Prostate Cancer") | cellmatch
obj <- findmarkergene(object = obj, if_use_custom_marker = TRUE, marker = cellmatch_new)
obj <- findcelltype(obj)
```

5. ShinySC

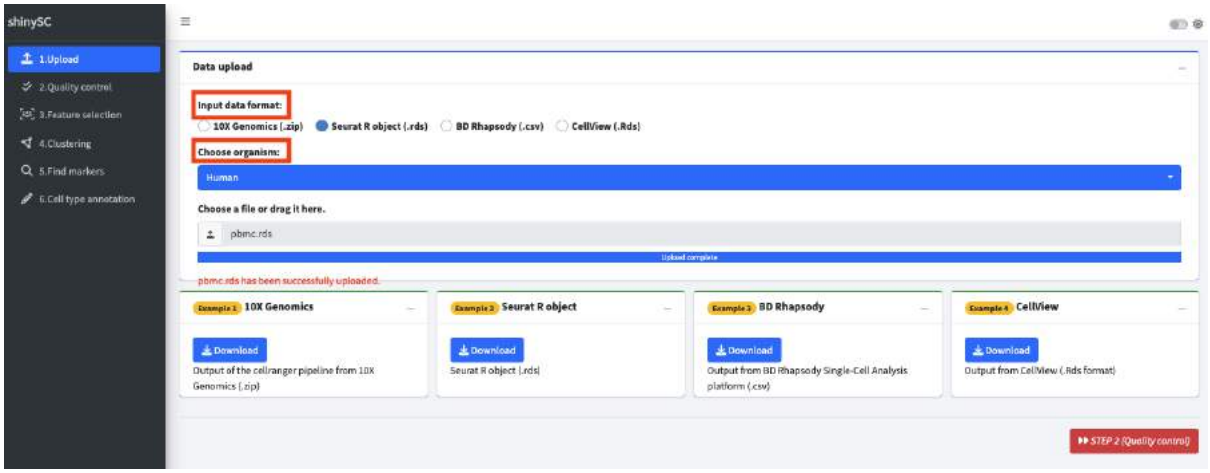
5-1 Upload

5-1-1 Input format:

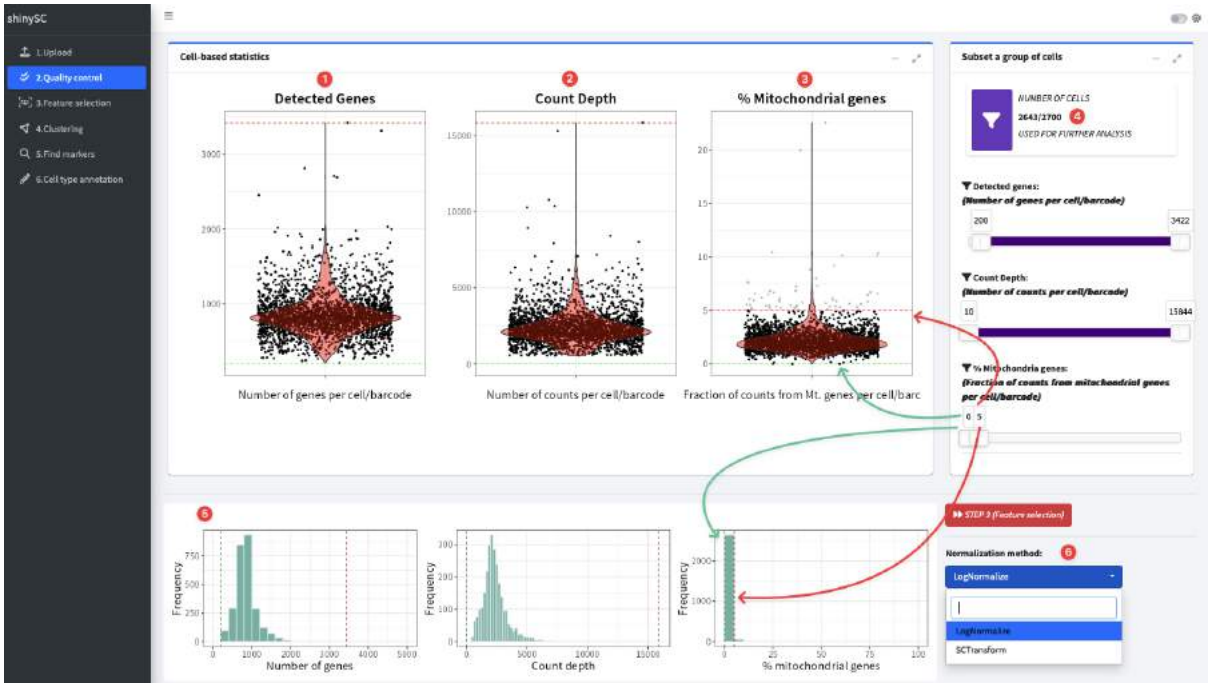
- **10X Genomics (.zip)**
- **Seurat R object (.rds)**
- **BD Rhapsody (.csv)**
- **CellView (.Rds)**

5-1-2 Supported organisms:

- **Human**
- **Mouse**

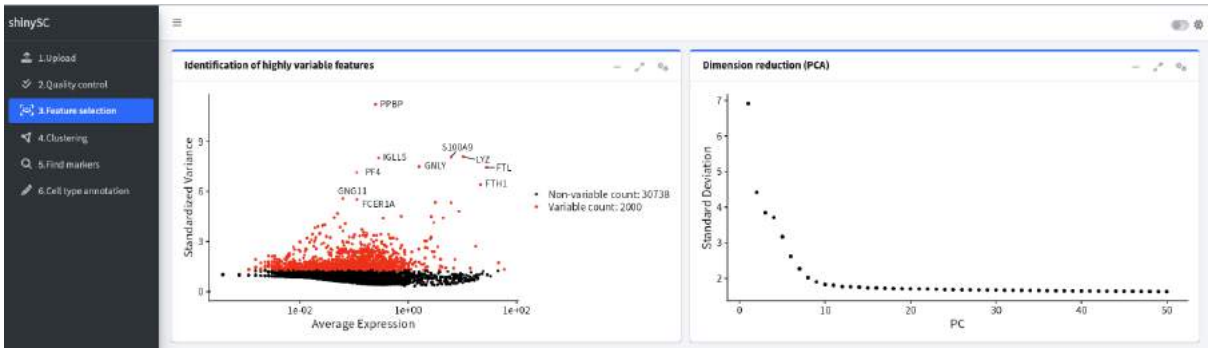


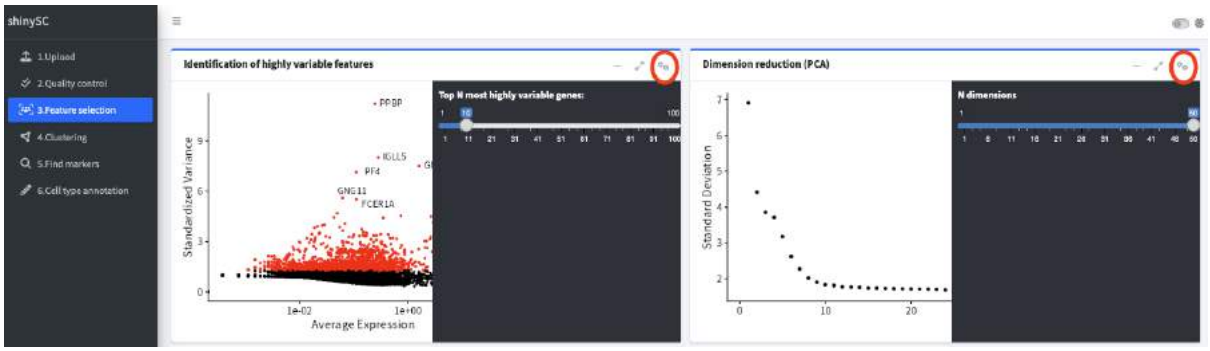
5-2 Quality control



5-3 Feature selection

5-3-1 Identification of highly variable features & Dimension reduction (PCA)



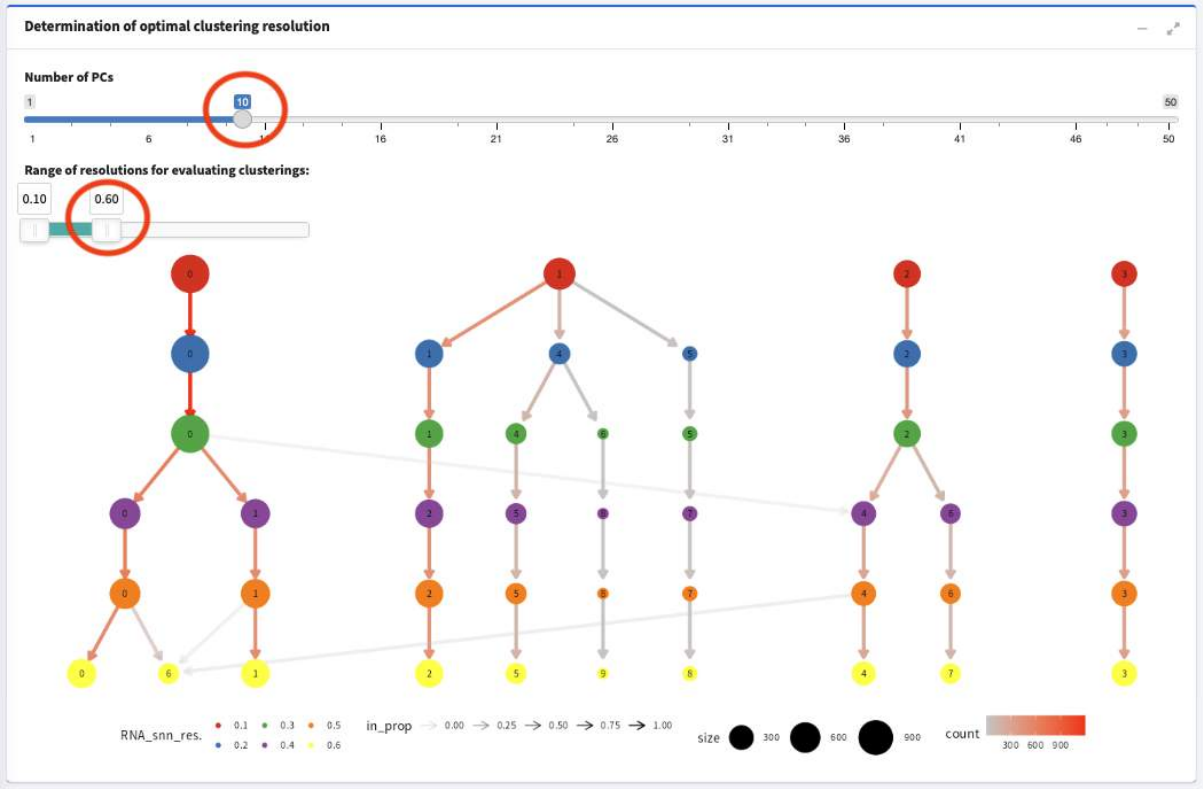


5-3-2 Determination of optimal clustering resolution

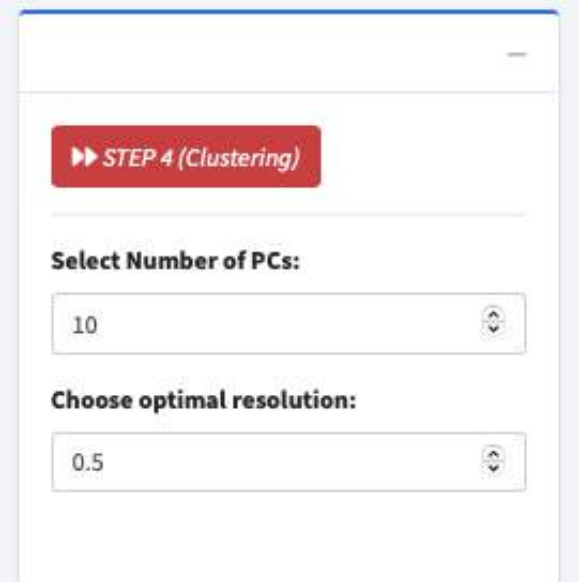
Tools and techniques for single-cell RNA sequencing data

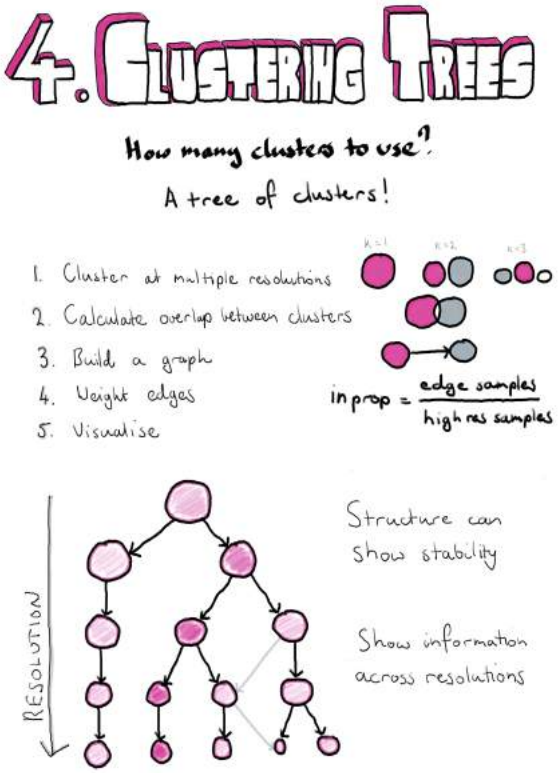
https://lazappi.github.io/phd-thesis/4-clust-trees.html

Clustering trees_a visualization for evaluating clusterings at multiple resolutions.pdf



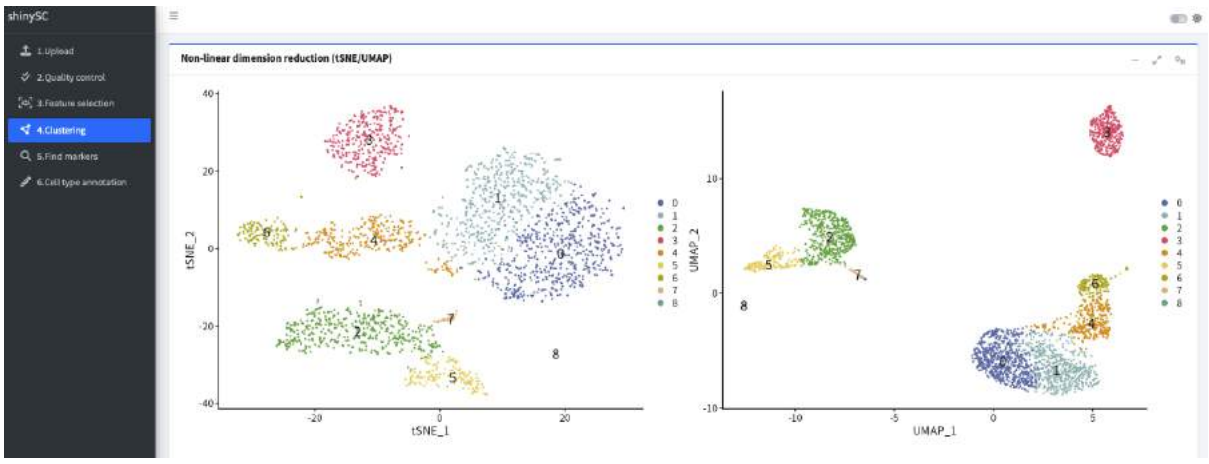
5-3-2 Define optimal resolution

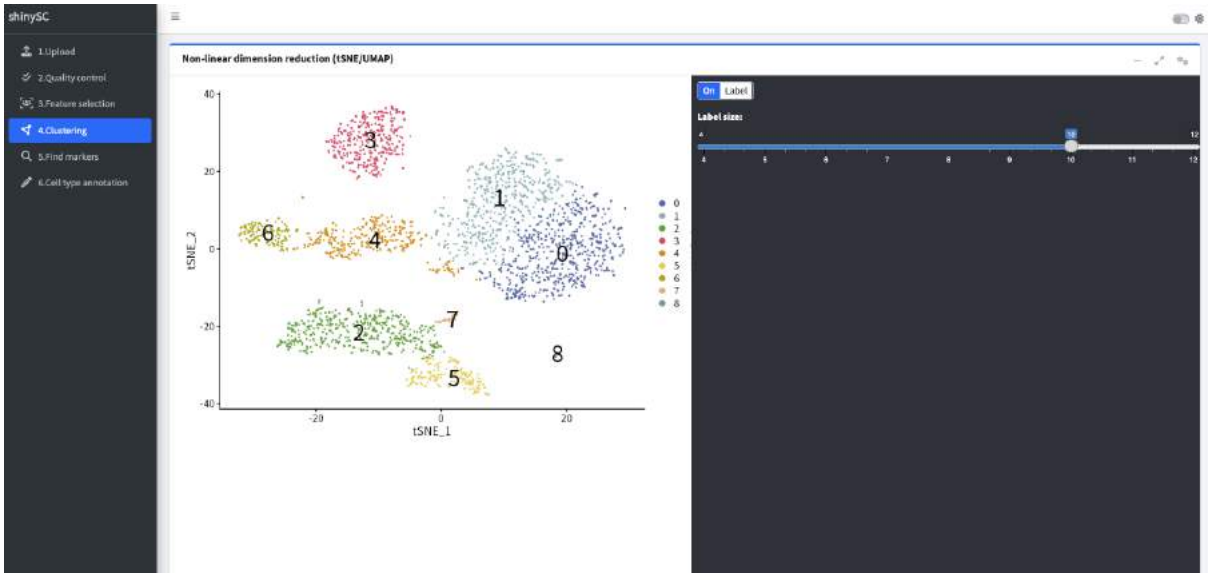




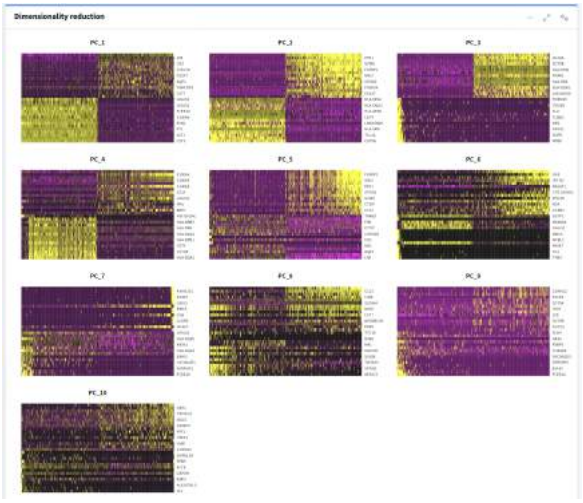
5-4. Clustering

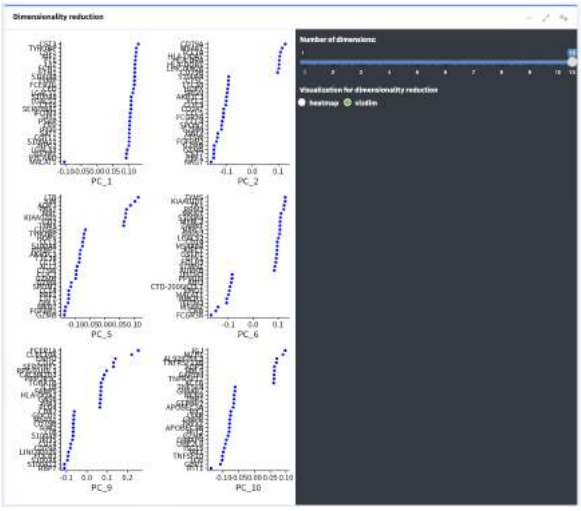
5-4-1 Non-linear dimension reduction





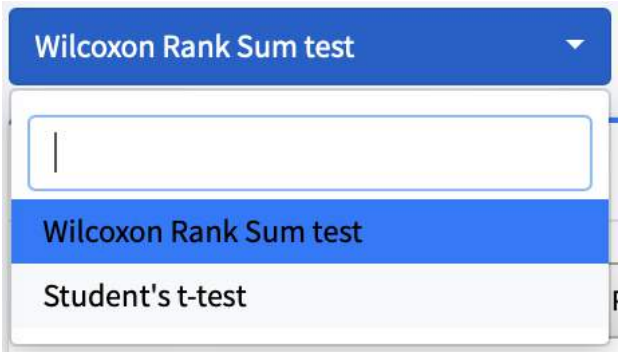
5-4-2 Heatmap & Vizdim



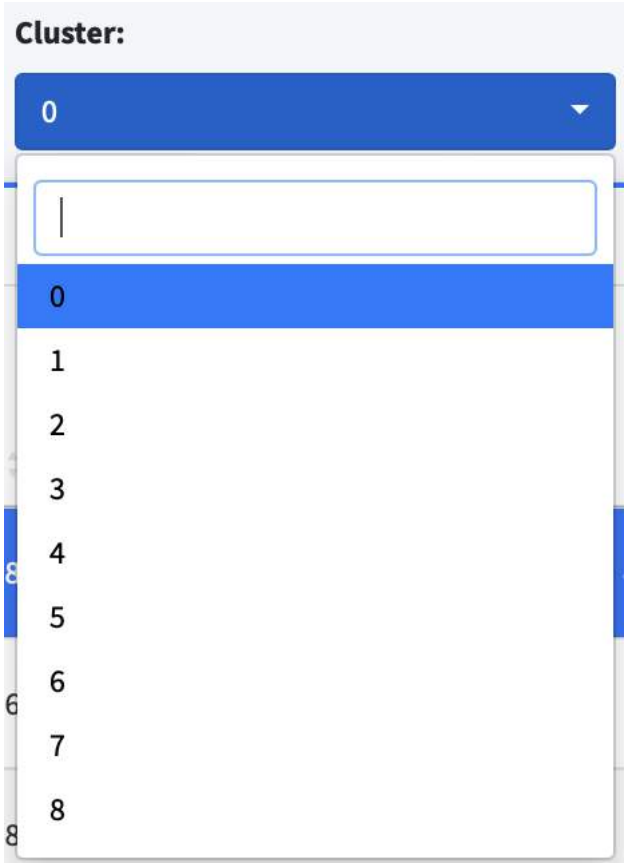


5-5 Find markers

▼ **Differential expression test**

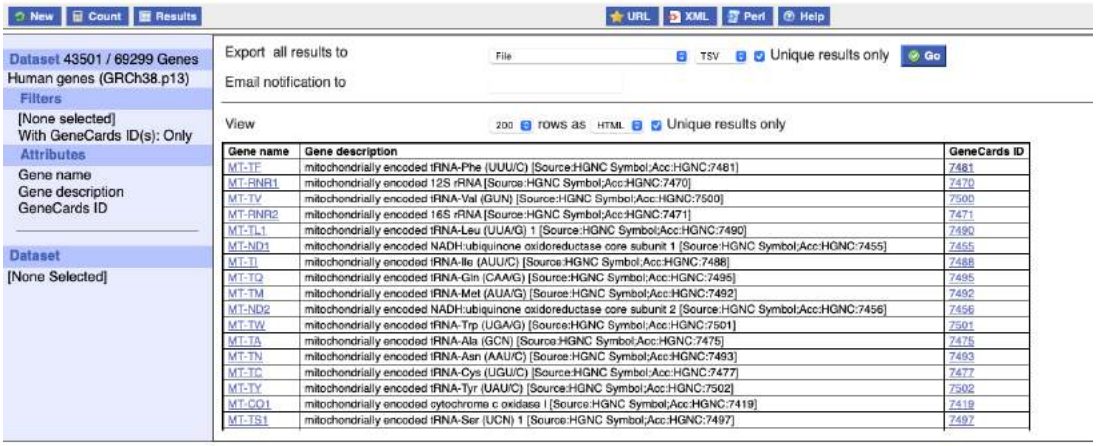


▼ **Cluster**



▼ **Gene description**

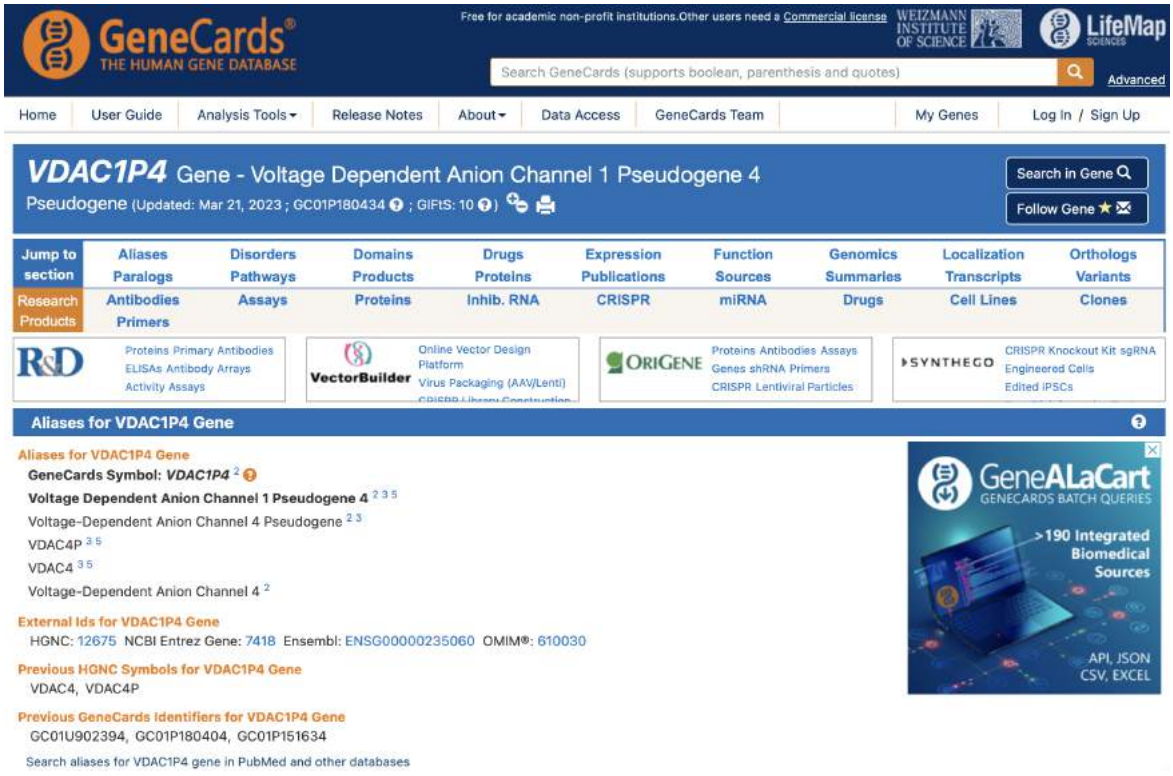
Biomart: Human (ok), Mouse (待確認Gene name格式)

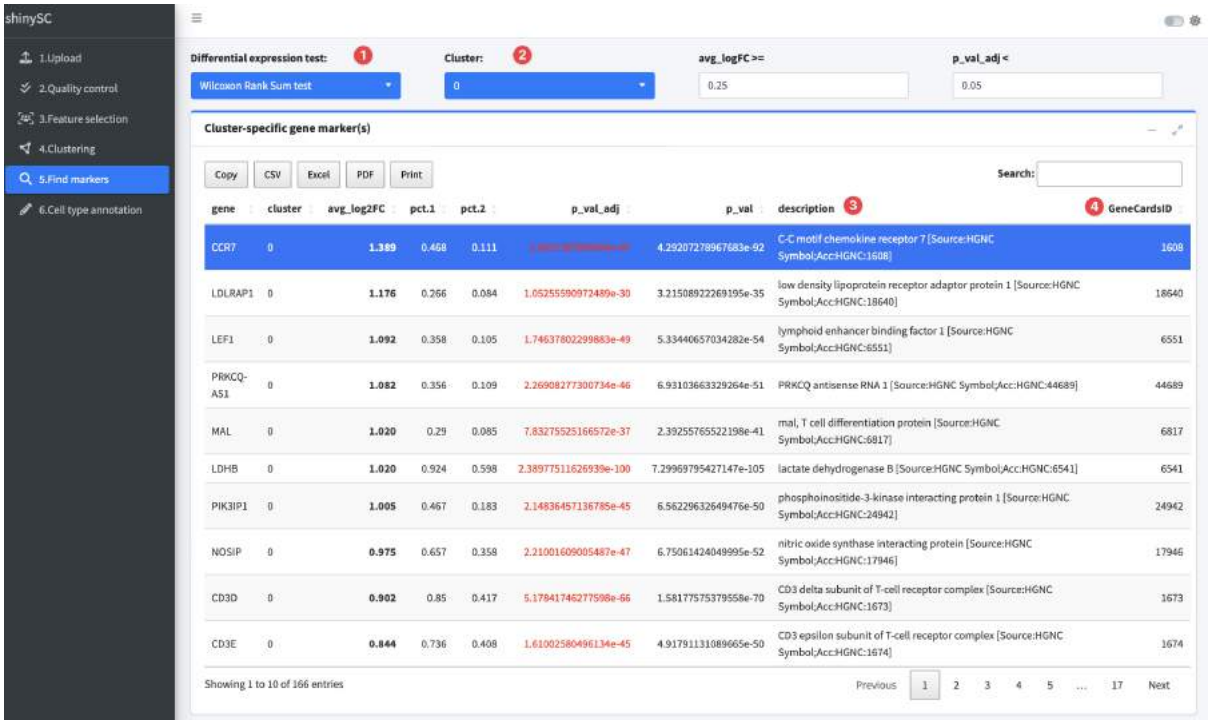


Gene name	Gene description	GeneCards ID
MT-TF	mitochondrially encoded tRNA-Phe (UUU/C) [Source:HGNC Symbol;Acc:HGNC:7481]	7481
MT-RNR1	mitochondrially encoded 12S rRNA [Source:HGNC Symbol;Acc:HGNC:7470]	7470
MT-TV	mitochondrially encoded tRNA-Val (GUN) [Source:HGNC Symbol;Acc:HGNC:7500]	7500
MT-RNR2	mitochondrially encoded 16S rRNA [Source:HGNC Symbol;Acc:HGNC:7471]	7471
MT-TL1	mitochondrially encoded tRNA-Leu (UUA/G) 1 [Source:HGNC Symbol;Acc:HGNC:7490]	7490
MT-ND1	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 1 [Source:HGNC Symbol;Acc:HGNC:7455]	7455
MT-TI	mitochondrially encoded tRNA-Ile (AUU/C) [Source:HGNC Symbol;Acc:HGNC:7488]	7488
MT-TQ	mitochondrially encoded tRNA-Gln (CAA/G) [Source:HGNC Symbol;Acc:HGNC:7495]	7495
MT-TM	mitochondrially encoded tRNA-Met (AUA/G) [Source:HGNC Symbol;Acc:HGNC:7492]	7492
MT-ND2	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 2 [Source:HGNC Symbol;Acc:HGNC:7456]	7456
MT-TW	mitochondrially encoded tRNA-Trp (UGA/G) [Source:HGNC Symbol;Acc:HGNC:7501]	7501
MT-TA	mitochondrially encoded tRNA-Ala (GCN) [Source:HGNC Symbol;Acc:HGNC:7475]	7475
MT-TN	mitochondrially encoded tRNA-Asn (AAU/C) [Source:HGNC Symbol;Acc:HGNC:7493]	7493
MT-TC	mitochondrially encoded tRNA-Cys (UGU/C) [Source:HGNC Symbol;Acc:HGNC:7477]	7477
MT-TY	mitochondrially encoded tRNA-Tyr (UAU/C) [Source:HGNC Symbol;Acc:HGNC:7502]	7502
MT-CO1	mitochondrially encoded cytochrome c oxidase I [Source:HGNC Symbol;Acc:HGNC:7419]	7419
MT-TS1	mitochondrially encoded tRNA-Ser (UCN) 1 [Source:HGNC Symbol;Acc:HGNC:7497]	7497

▼ **GeneCards**

(GeneCardsID → GeneCards web site 連結待建立)





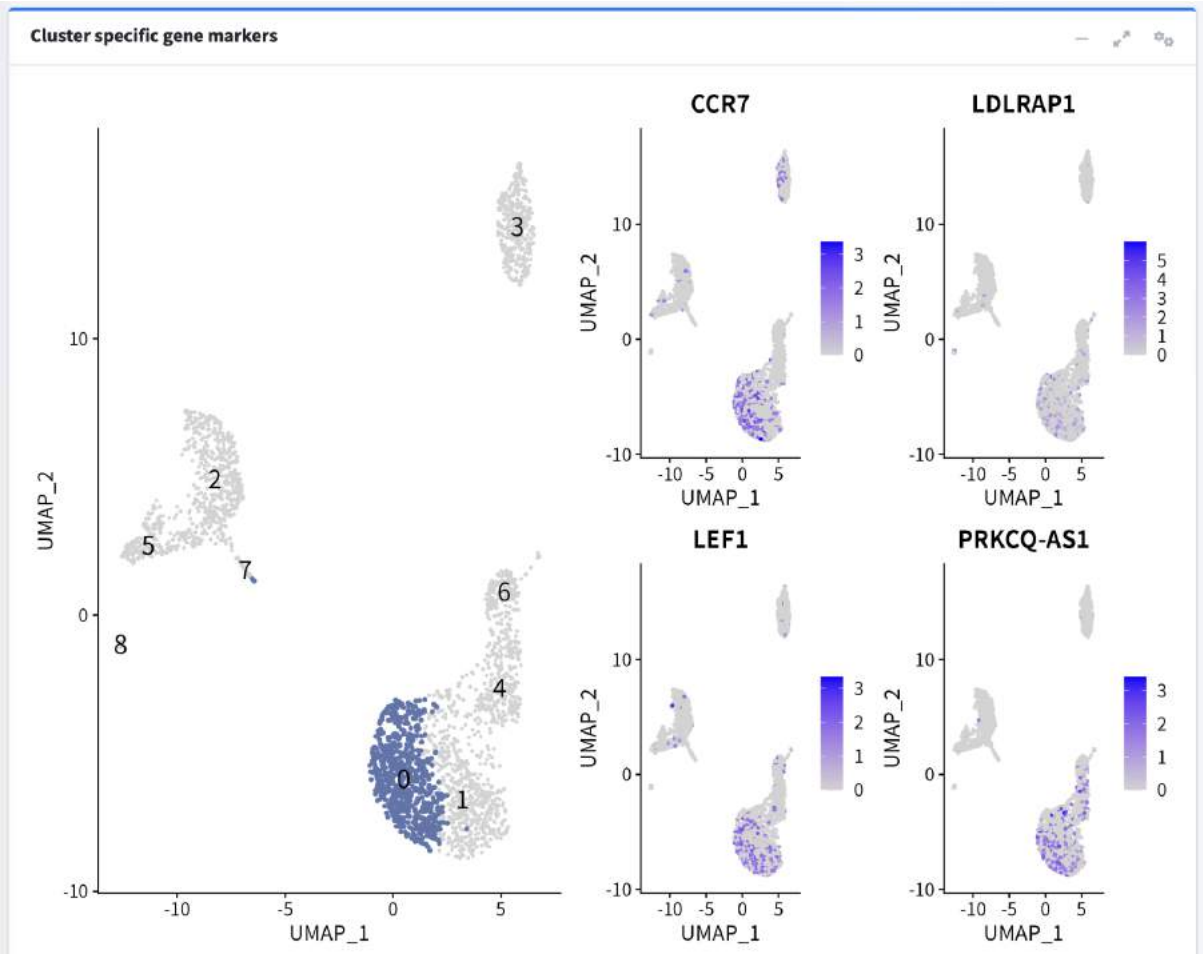
Identification of Cluster-specific gene markers

Cluster-specific gene marker(s)

Copy CSV Excel PDF Print Search:

gene	cluster	avg_log2FC	pct.1	pct.2	p_val_adj	p_val	description
CCR7	0	**1.389**	0.468	0.111	[illegible]	4.29207278967683e-92	C-C motif chemokine receptor 7 [Source:HGNC Symbol;Acc:HGNC:1608]
LDLRAP1	0	**1.176**	0.266	0.084	[illegible]	3.21508922269195e-35	low density lipoprotein receptor adaptor protein 1 [Source:HGNC Symbol;Acc:HGNC:18640]
LEF1	0	**1.092**	0.358	0.105	[illegible]	5.33440657034282e-54	lymphoid enhancer binding factor 1 [Source:HGNC Symbol;Acc:HGNC:6551]
PRKCQ-AS1	0	**1.082**	0.356	0.109	[illegible]	6.93103663329264e-51	PRKCQ antisense RNA 1 [Source:HGNC Symbol;Acc:HGNC:44689]
MAL	0	**1.020**	0.29	0.085	7.83275525166572e-37	2.39255765522198e-41	mal, T cell differentiation protein [Source:HGNC Symbol;Acc:HGNC:6817]
LDHB	0	**1.020**	0.924	0.598	2.38977511626939e-100	7.29969795427147e-105	lactate dehydrogenase B [Source:HGNC Symbol;Acc:HGNC:6541]
PIK3IP1	0	**1.005**	0.467	0.183	2.14836457136785e-45	6.56229632649476e-50	phosphoinositide-3-kinase interacting protein 1 [Source:HGNC Symbol;Acc:HGNC:24942]
NOSIP	0	**0.975**	0.657	0.358	2.21001609005487e-47	6.75061424049995e-52	nitric oxide synthase interacting protein [Source:HGNC Symbol;Acc:HGNC:17946]
CD3D	0	**0.902**	0.85	0.417	5.17841746277598e-66	1.58177575379558e-70	CD3 delta subunit of T-cell receptor complex [Source:HGNC Symbol;Acc:HGNC:1673]
CD3E	0	**0.844**	0.736	0.408	1.61002580496134e-45	4.91791131089665e-50	CD3 epsilon subunit of T-cell receptor complex [Source:HGNC Symbol;Acc:HGNC:1674]

Showing 1 to 10 of 166 entries Previous 1 2 3 4 5 … 17 Next



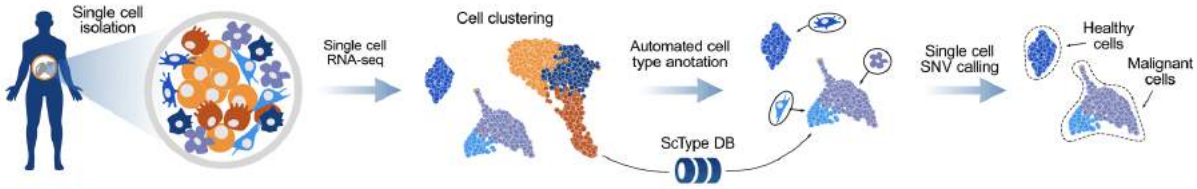
5-6. Automated cell-type annotation

5-6-1 ScType

Fully-automated and ultra-fast cell-type identification using specific marker combinations from single-cell transcriptomic data

Aleksandr Ianevski, Anil K. Giri & Tero Aittokallio

Nature Communications **13**, Article number: 1246 (2022) | Cite this article



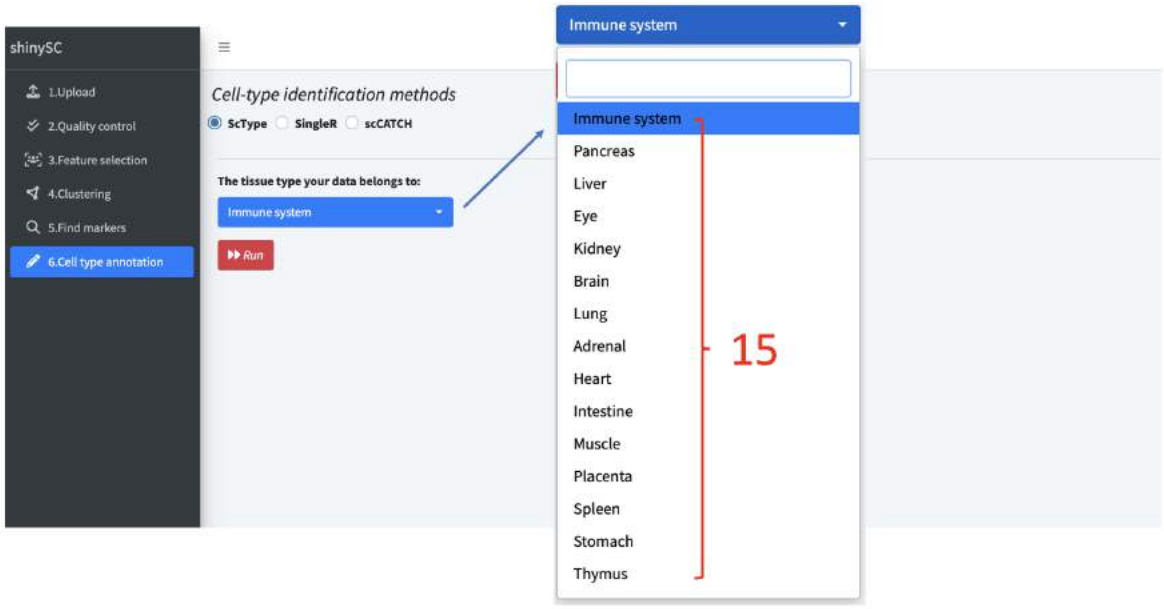
s41467-022-28803-w.pdf

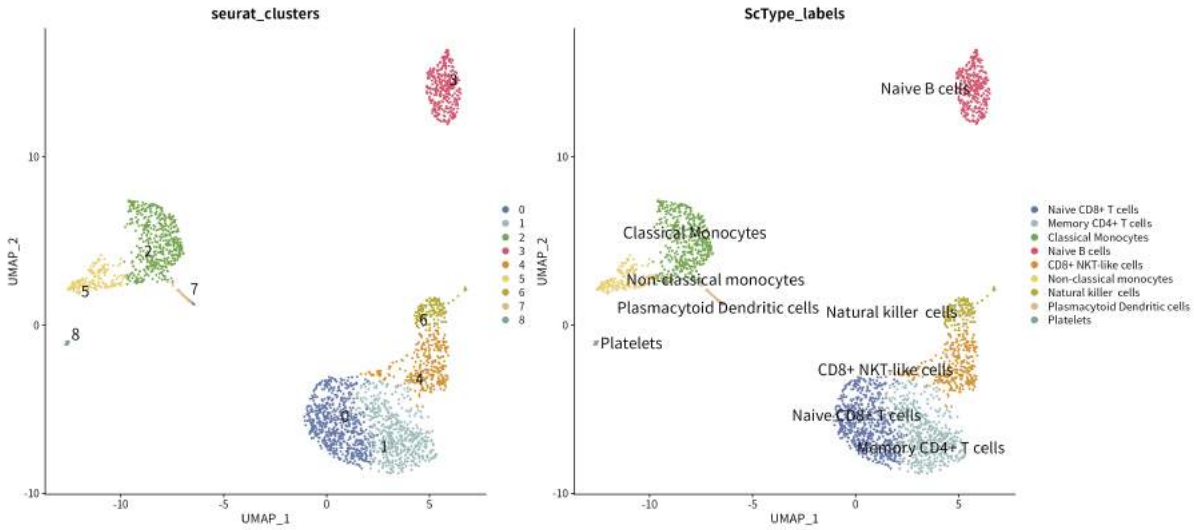
The widely applicable method is deployed both as **an interactive web-tool (https://sctype.app)**, and as **an open-source R-package**.

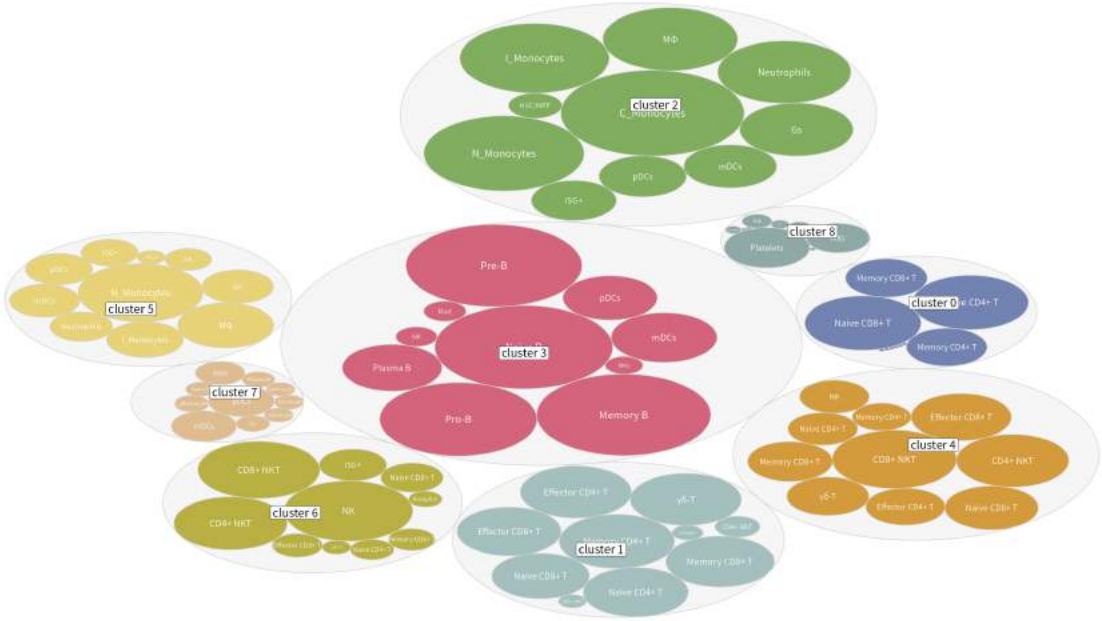
https://github.com/IanevskiAleksandr/sc-type

The ScType tool is currently undergoing an update and is expected to be back up and running within the next few days!

Please use our R-based implementation in the meanwhile:
https://github.com/IanevskiAleksandr/sc-type


shinySC
1.Upload
2.Quality control
3.Feature selection
4.Clustering
5.Find markers
6.Cell type annotation
Cell-type identification methods
ScType
SingleR
scCATCH
The tissue type your data belongs to:
Immune system
Run
Immune system
Pancreas
Liver
Eye
Kidney
Brain
Lung
Adrenal
Heart
Intestine
Muscle
Placenta
Spleen
Stomach
Thymus
15


seurat_clusters
UMAP_2
UMAP_1
ScType_labels
Naive B cells
Classical Monocytes
Non-classical monocytes
Plasmacytoid Dendritic cells
Natural killer cells
Platelets
CD8+ NKT-like cells
Naive CD8+ T cells
Memory CD4+ T cells
Naive CD8+ T cells
Memory CD4+ T cells
Classical Monocytes
Naive B cells
CD8+ NKT-like cells
Non-classical monocytes
Natural killer cells
Plasmacytoid Dendritic cells
Platelets



5-6-2 SingleR

Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage

Dvir Aran, Agnieszka P. Looney, Leqian Liu, Esther Wu, Valerie Fong, Austin Hsu, Suzanna Chak, Ram P. Naikawadi, Paul J. Wolters, Adam R. Abate, Atul J. Butte & Mallar Bhattacharya

Nature Immunology **20**, 163–172 (2019) | Cite this article

s41590-018-0276-y.pdf

SingleR

Performs unbiased cell type recognition from single-cell RNA sequencing data, by leveraging reference transcriptomic datasets of pure cell types to infer the cell of origin of each single cell independently.

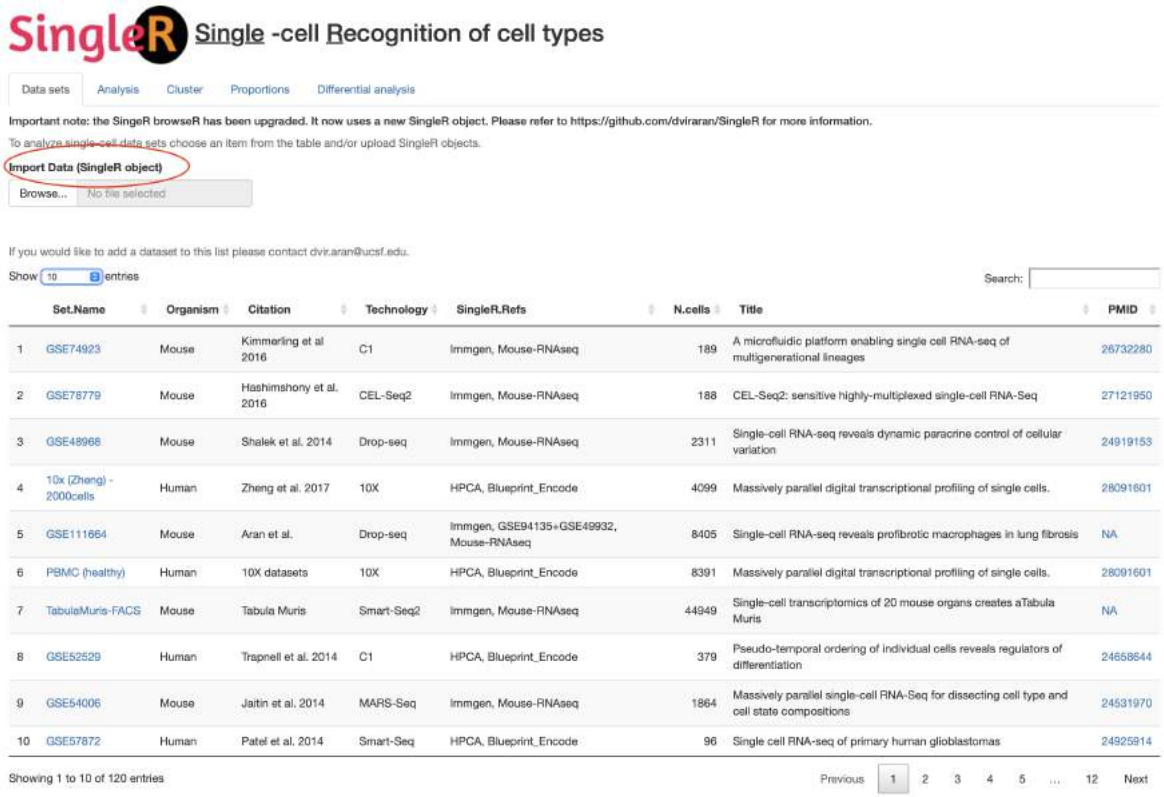
https://bioconductor.org/packages/release/bioc/html/SingleR.html

Bioconducto
OPEN SOURCE SOFTWARE FOR BIOINFORMA

singler - Default

https://comphealth.ucsf.edu/app/singler

▼ **singler web application**

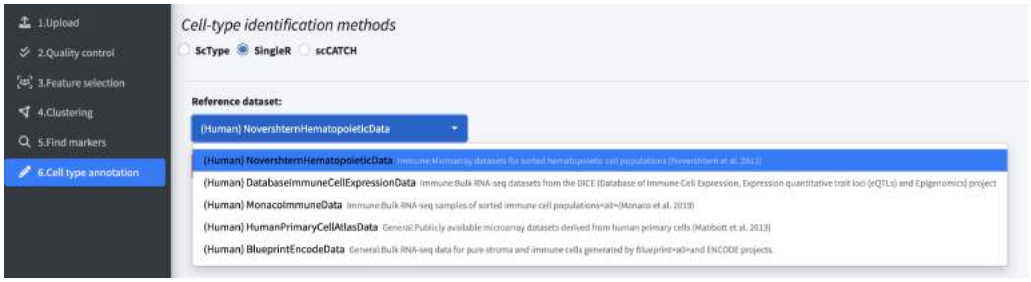


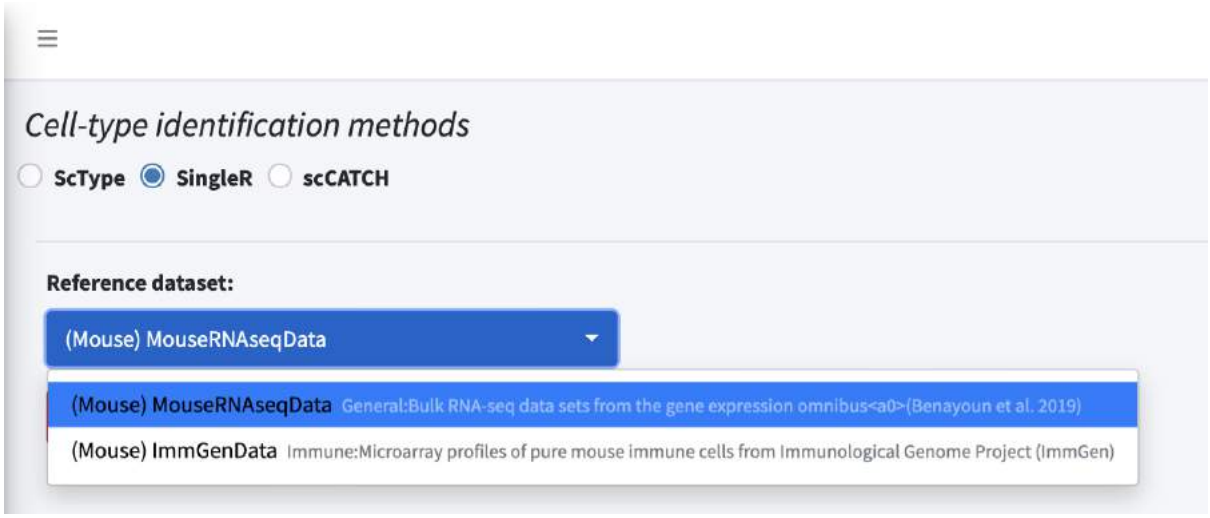
Reference Index for cell type

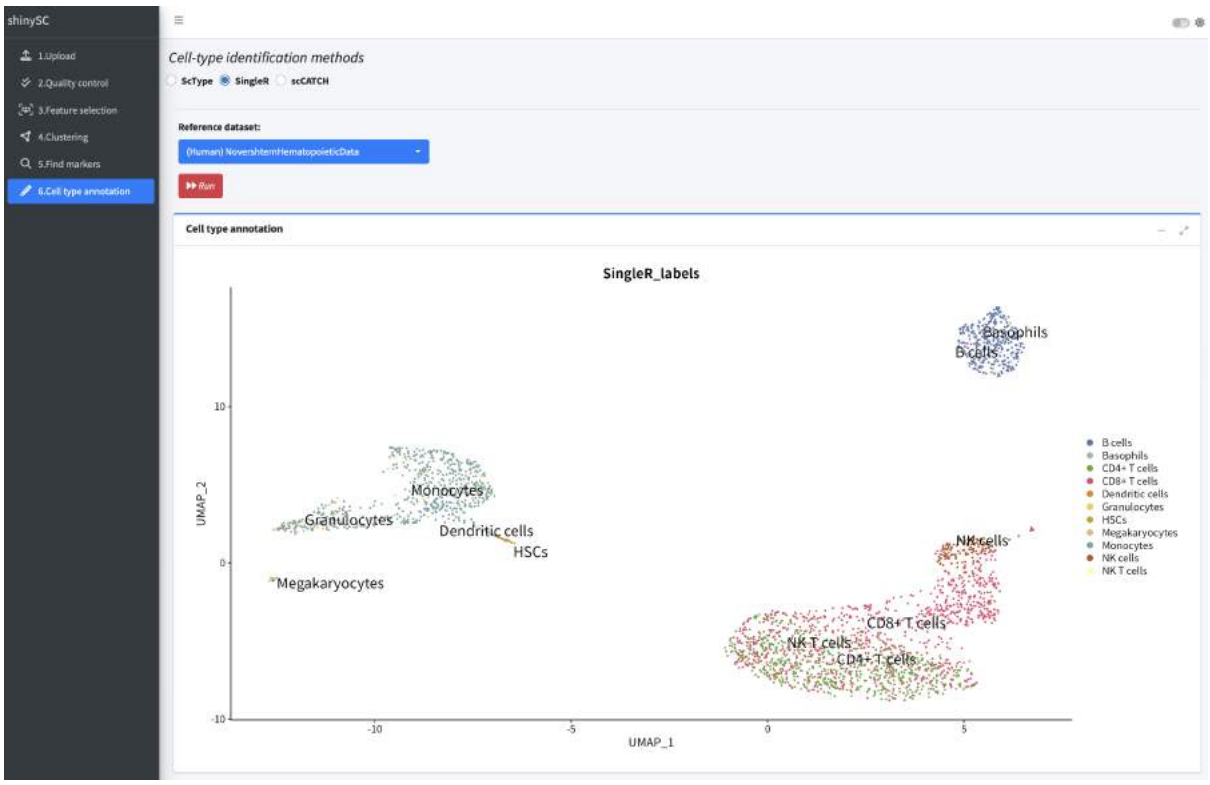
```
biocmanager::install("celldex")
```

- 建立local databases

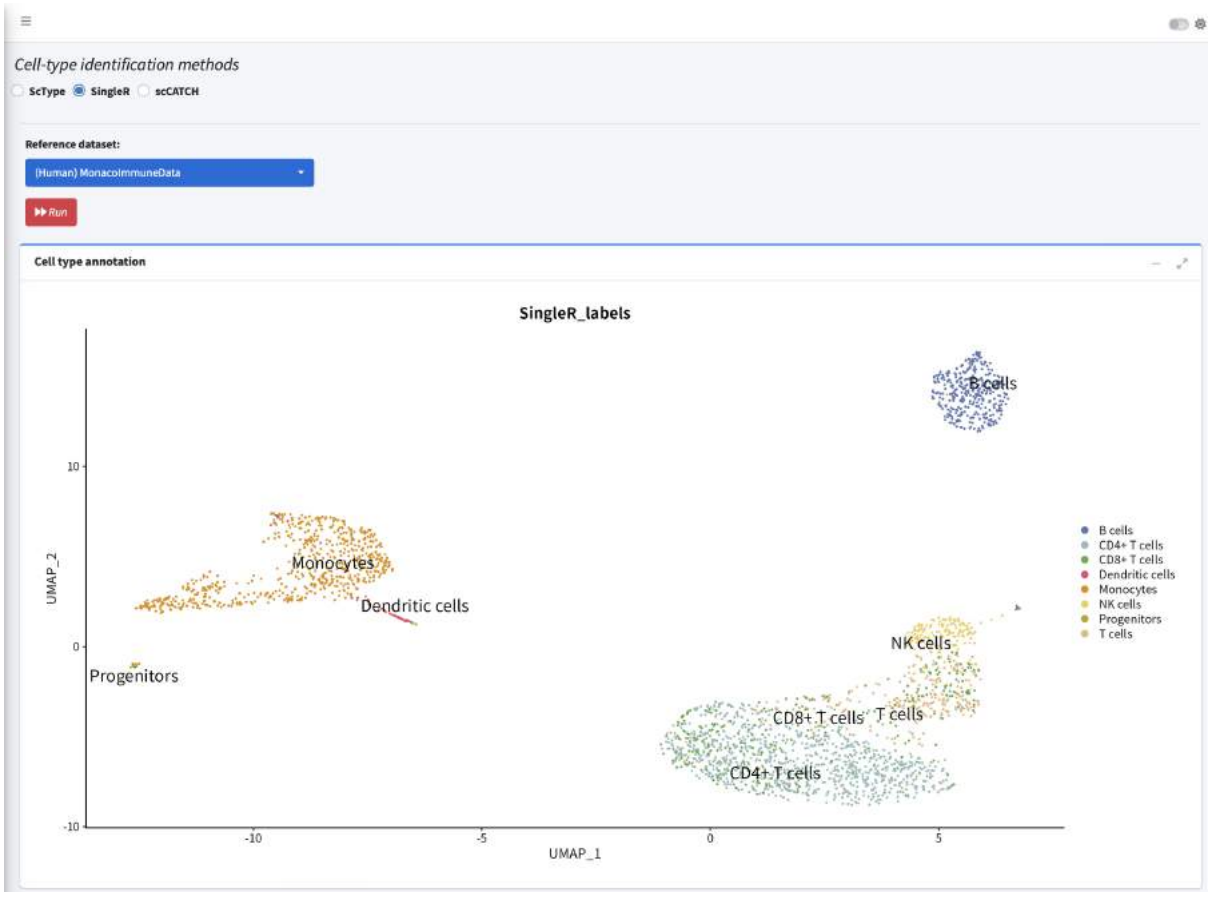
```
ref = celldex::DatabaseImmuneCellExpressionData()
saveRDS(ref,"DatabaseImmuneCellExpressionData.rds")
```




Cell-type identification methods
ScType
SingleR
scCATCH
Reference dataset:
(Mouse) MouseRNAseqData
(Mouse) MouseRNAseqData General:Bulk RNA-seq data sets from the gene expression omnibus<a0>(Benayoun et al. 2019)
(Mouse) ImmGenData Immune:Microarray profiles of pure mouse immune cells from Immunological Genome Project (ImmGen)


shinySC
1.Upload
2.Quality control
3.Feature selection
4.Clustering
5.Find markers
6.Cell type annotation
Cell-type identification methods
ScType
SingleR
scCATCH
Reference dataset:
(Human) NovershternHematopoieticData
Run
Cell type annotation
SingleR_labels
Basophils
B cells
Monocytes
Granulocytes
Dendritic cells
HSCs
Megakaryocytes
NK cells
CD8+ T cells
NK T cells
CD4+ T cells
B cells
Basophils
CD4+ T cells
CD8+ T cells
Dendritic cells
Granulocytes
HSCs
Megakaryocytes
Monocytes
NK cells
NK T cells
UMAP_2
UMAP_1
10
0
-10
-10
-5
0
5


Cell-type identification methods
ScType
SingleR
scCATCH
Reference dataset:
(Human) BlueprintEncodeData
Run
Cell type annotation
SingleR_labels
B-cells
Monocytes
HSC
NK cells
CD8+ T-cells
CD4+ T-cells
UMAP_2
UMAP_1
B-cells
CD4+ T-cells
CD8+ T-cells
HSC
Monocytes
NK cells


Cell-type identification methods
ScType
SingleR
scCATCH
Reference dataset:
(Human) MonacoImmuneData
Run
Cell type annotation
SingleR_labels
B cells
Monocytes
Dendritic cells
Progenitors
NK cells
CD8+ T cells
T cells
CD4+ T cells
UMAP_2
UMAP_1
B cells
CD4+ T cells
CD8+ T cells
Dendritic cells
Monocytes
NK cells
Progenitors
T cells

5-6-3

scCATCH: Automatic Annotation on Cell Types of Clusters from Single-Cell RNA Sequencing Data

Xin Shao [1], Jie Liao [1], Xiaoyan Lu [1], Rui Xue [1], Ni Ai [1], Xiaohui Fan [2]

Affiliations + expand

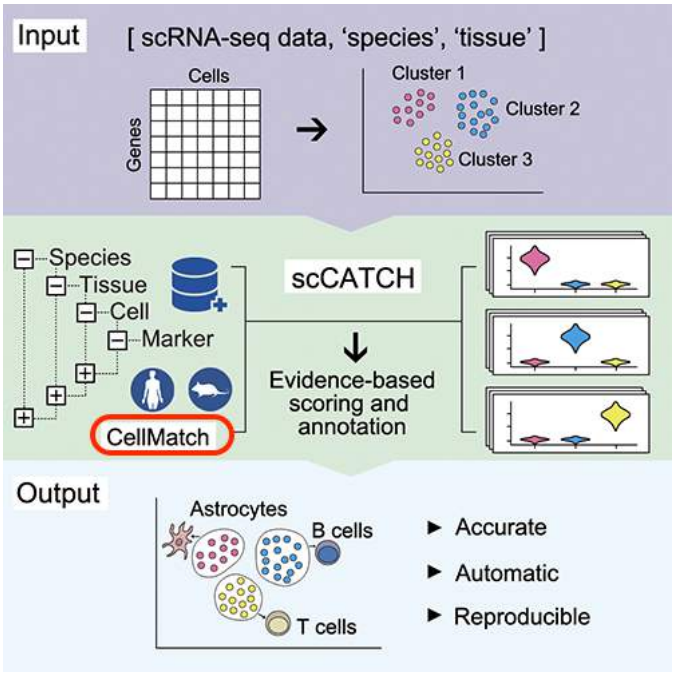
PMID: 32062421 PMCID: PMC7031312 DOI: 10.1016/j.isci.2020.100882

main.pdf

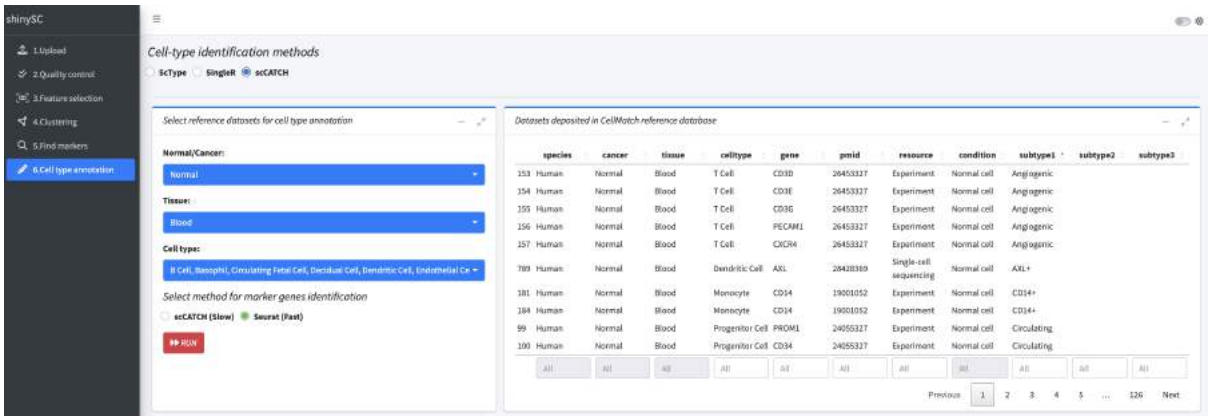
https://github.com/ZJUFanLab/scCATCH

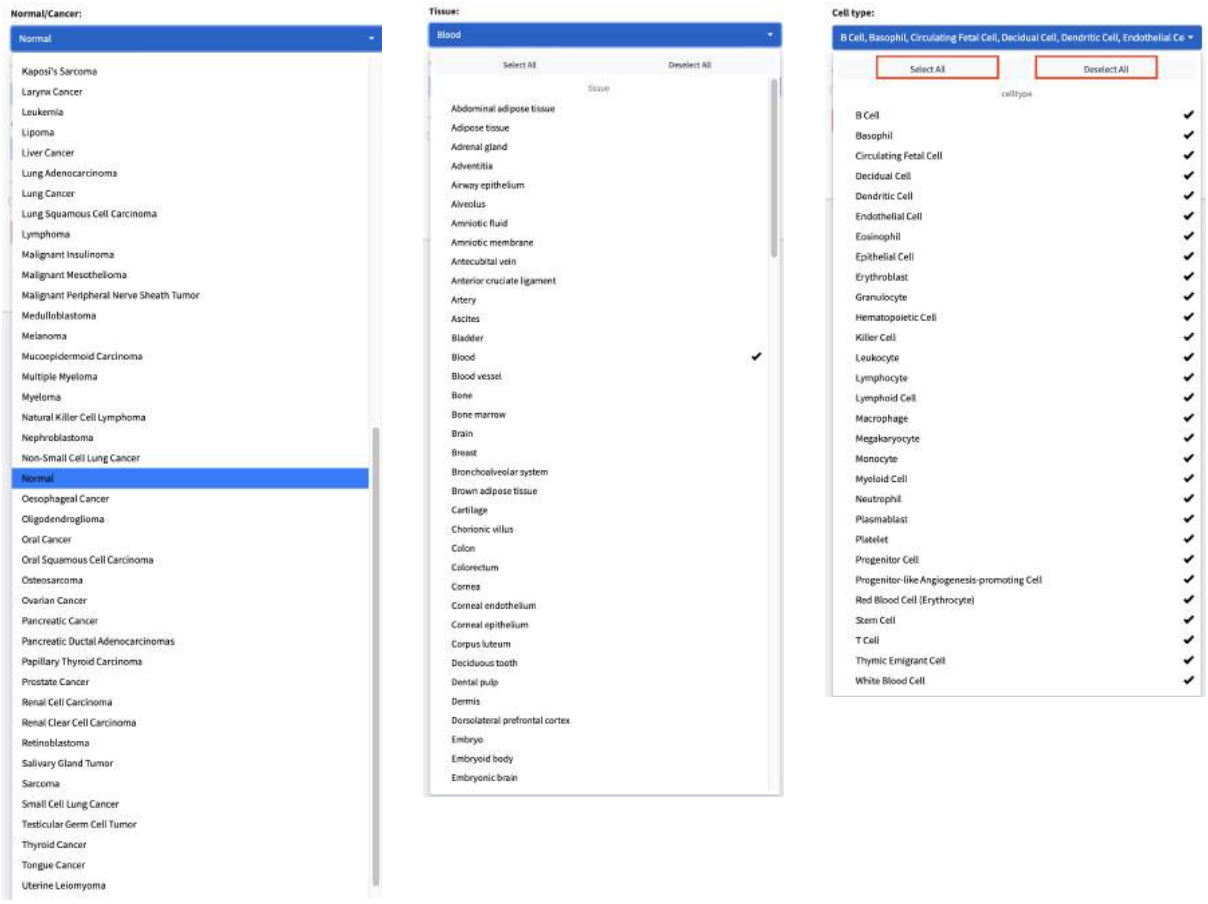
Knowledge in **CellMatch reference database** was derived from various resources, such as **CellMarker (Zhang et al., 2019b)**, **MCA (Han et al., 2018)**, **CancerSEA (Yuan et al., 2019)**, and the **CD Marker Handbook**.

a panel of 353 cell types and related 686 subtypes associated with **184 tissue types**, **20,792 cell-specific marker genes**, and **2,097 references of humans and mice** were introduced into scCATCH as the reference database.

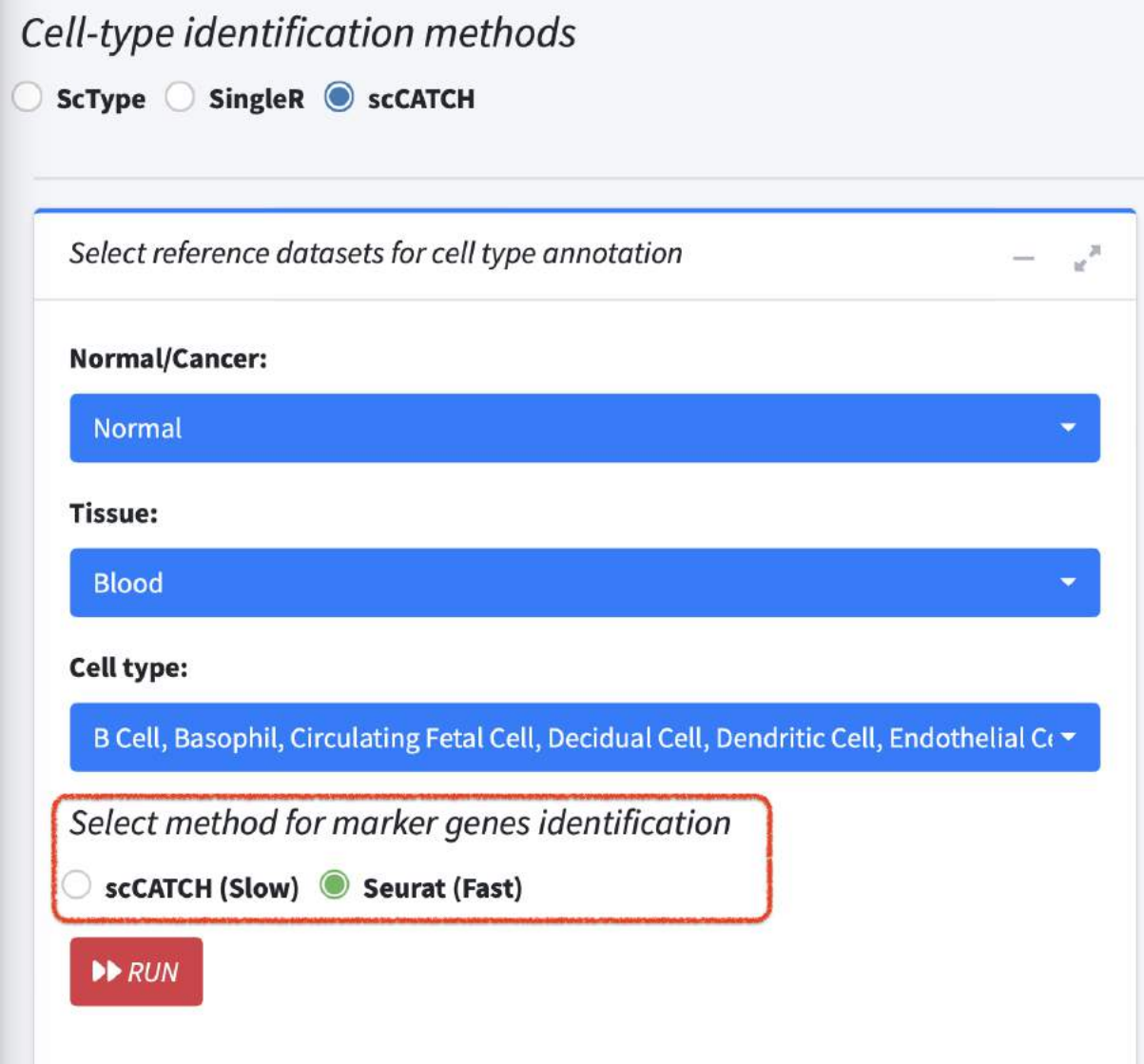


- Cascade filters for CellMatch reference database





- The differences between Seurat and scCATCH



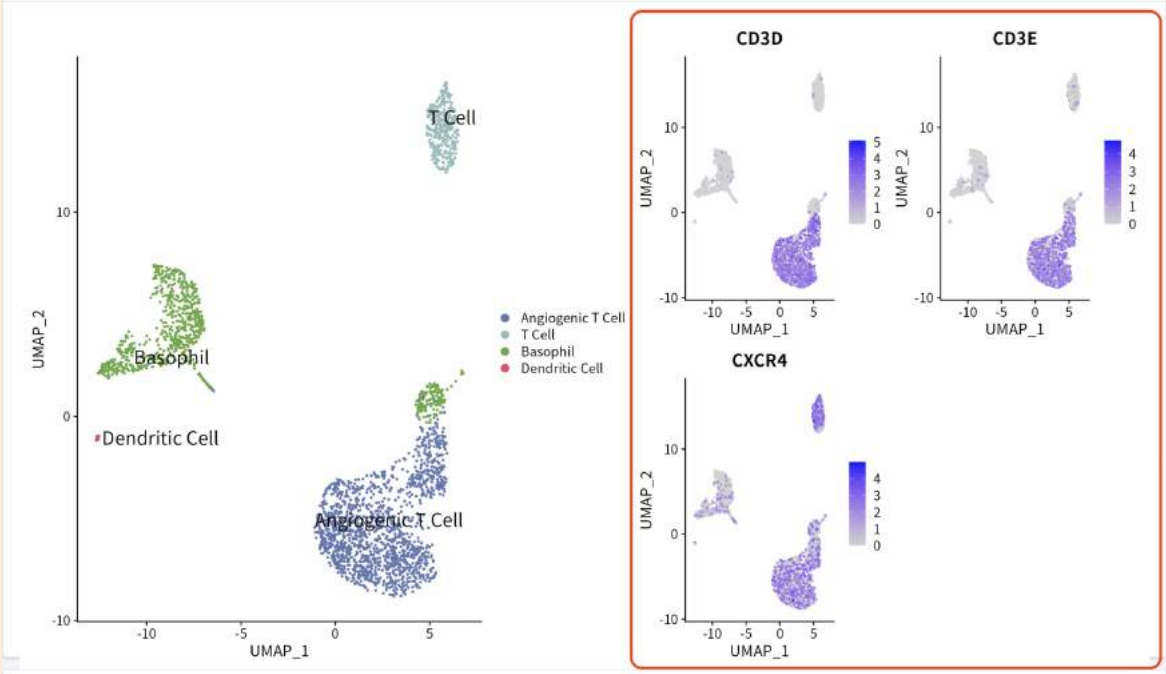
According to page 5 of the PDF, Seurat and scCATCH differ in their approach to finding marker genes for clusters. Seurat uses a one-against-all approach, potentially generating a set of pseudo cluster potential marker genes (highly expressed in at least two clusters). On the other hand, scCATCH carries out paired comparisons to identify differentially expressed genes in only one specific cluster to ensure accuracy in matching the CellMatch database. The cluster potential marker genes identified through scCATCH analysis usually were a subset of genes determined via Seurat. However, an increased number of cluster potential marker genes did not benefit cell annotation.

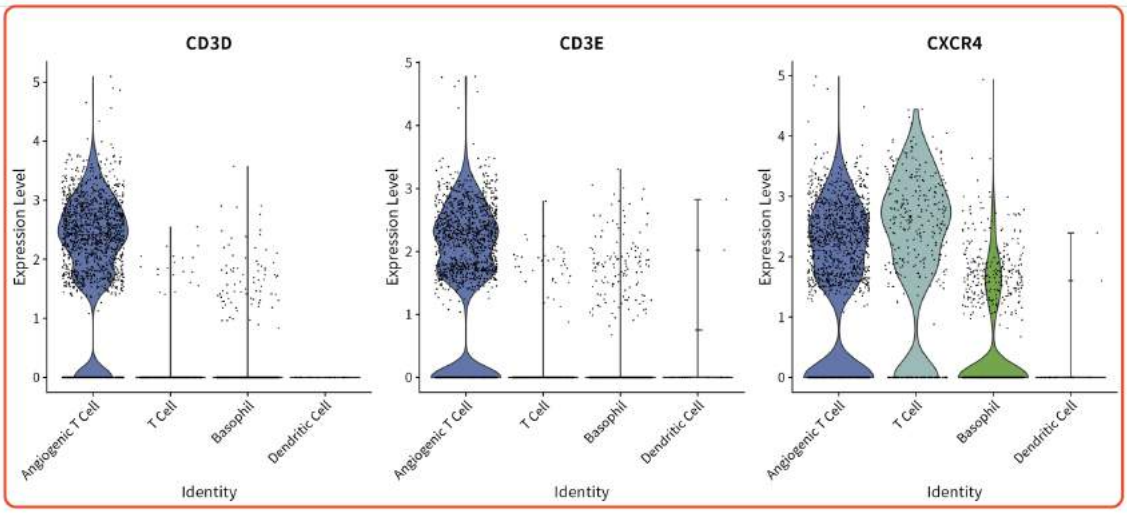
▼ **Seurat approach**

Show 5 entries Search:

cluster	cluster_marker	cell_type	celltype_score	celltype_related_marker	PMID
0	JUN, SELL, CXCR4, IL7R, LTB, GIMAP7, RGS10, CD3E, CD3D, EVL, JUNB, DNAJB1, PIK3IP1	Angiogenic T Cell	0.92	CD3D, CD3E, CXCR4	26453327
1	CD52, JUN, CD2, TXNIP, S100A10, CD48, BIN1, CXCR4, IL7R, DUSP1, HLA-E, LTB, GIMAP7, GIMAP4, CD99, PLP2, TSC22D3, ANXA1, IFITM1, PTPRCAP, CD3E, CD3D, SLC2A3, CD69, NAP1L1, ITM2B, EVL, CRIP1, JUNB	Angiogenic T Cell	0.92	CD3D, CD3E, CXCR4	26453327
2	ISG15, IFI6, CTSS, S100A10, S100A11, S100A9, S100A8, S100A4, CD48, ID2, TKT, ANXA5, DUSP1, LST1, AIF1, HLA-DPA1, HLA-DPB1, SNX3, ACTB, SAT1, TIMP1, SSR4, LY6E, FCN1, PSAP, FTH1, NEAT1, CASP4, CASP1, CD63, LYZ, NPC2, MT2A, ARRB2, TYROBP, FTL, TYMP, ITGB2	Basophil	0.88	CD63	28342911, 26184676, 21421855, 20558998, 19945674, 18817894, 14707480
3	CD52, SELL, CXCR4, PLAC8, LTB, HLA-DPA1, HLA-DPB1, SNX3, PTPRCAP, CD69	T Cell	0.75	CXCR4, SELL, CD69	26453327, 22697005, 28566371
4	CD2, ID2, DUSP2, IL7R, HLA-E, CD99, ANXA1, CTSW, PTPRCAP, CD3E, CD3D	Angiogenic T Cell	0.91	CD3D, CD3E	26453327

Showing 1 to 5 of 9 entries Previous 1 2 Next





▼ **scCATCH approach**

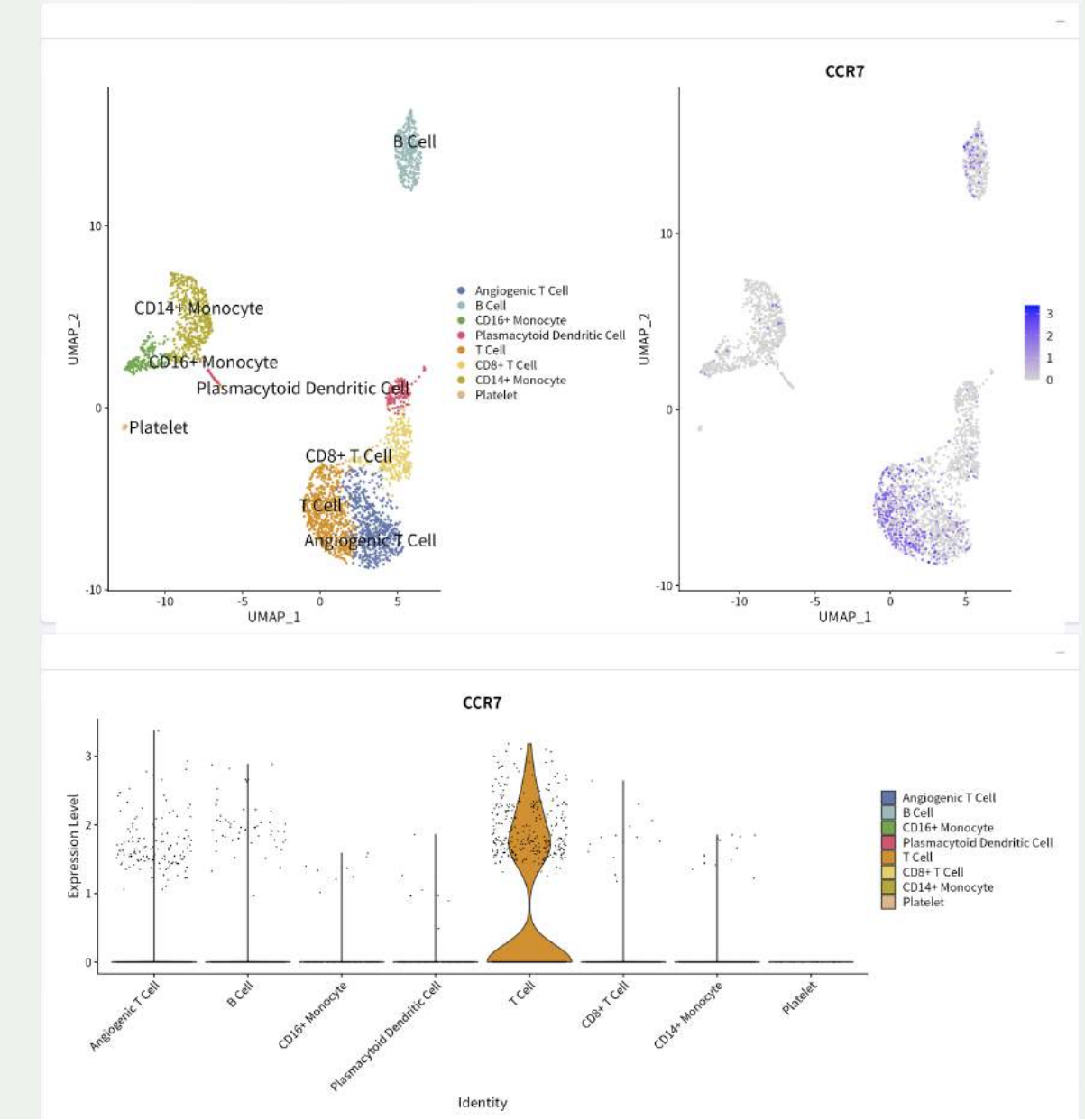
Show 5 entries

Search:

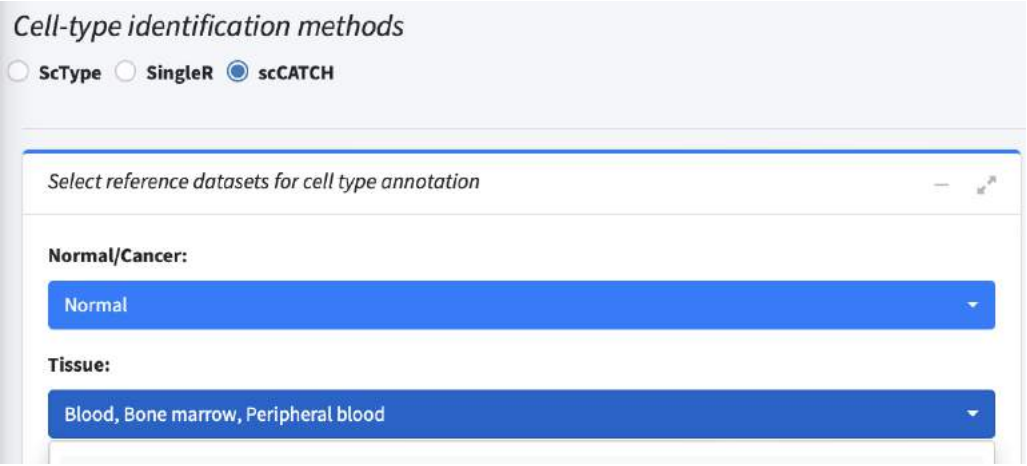
cluster	cluster_marker	cell_type	celltype_score	celltype_related_marker	PMID
0	CCR7	T Cell	0.5	CCR7	28929596
1	CD2, IL7R, LTB, GIMAP7, CD3E, CD3D	Angiogenic T Cell	0.91	CD3D, CD3E	26453327
2	S100A9, S100A12, S100A8, CD14, FCN1, ASGR1	CD14+ Monocyte	0.88	CD14	19001052
3	MS4A1, TCL1A, CD79B	B Cell	0.75	MS4A1	25254006, 6970772, CD Handbook
4	CD8A	CD8+ T Cell	0.8	CD8A	10754519, 10640724

Showing 1 to 5 of 9 entries

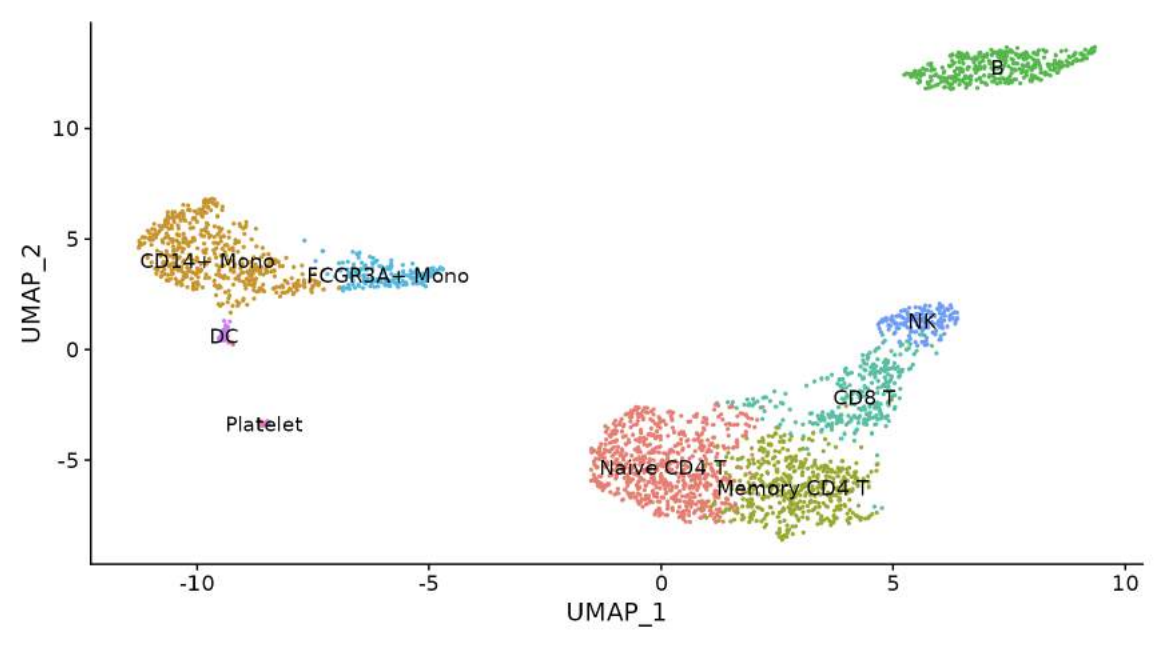
Previous 1 2 Next



💡 **Tissue: 'Blood','Peripheral blood','Bone marrow'**



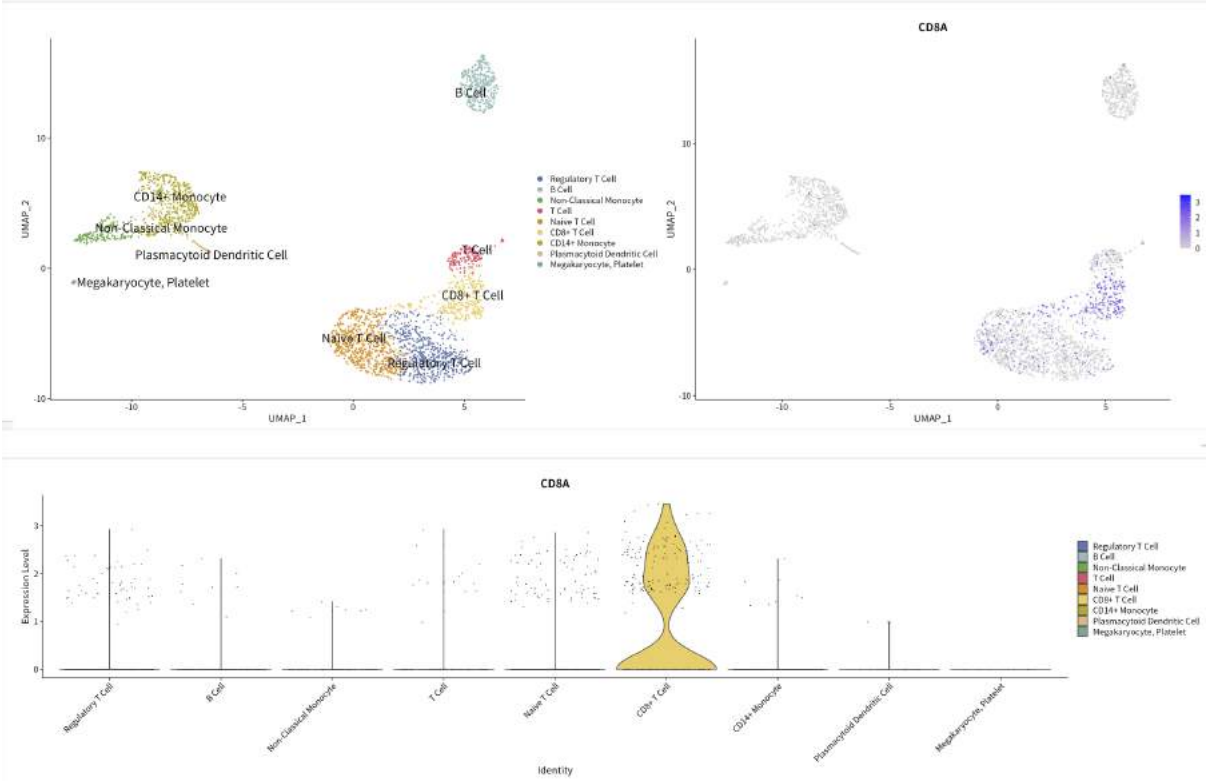
▼ **Seurat 提供範例**



Show 5 entries Search:

cluster	cluster_marker	cell_type	celltype_score	celltype_related_marker	PMID
0	CCR7	Naive T Cell	0.85	CCR7	23044634, 29361178, 28622514
1	CD2, IL7R, LTB, GIMAP7, CD3E, CD3D, LDHB	Regulatory T Cell	0.96	CD3D, CD3E, IL7R	28777444, 26049548, 23769051
2	S100A9, S100A12, S100A8, CD14, FCN1, ASGR1	CD14+ Monocyte	0.94	CD14	19001052, 29361178
3	MS4A1, TCL1A, ISG20, CD79B, FCER2, CD79A, CD37	B Cell	0.92	MS4A1, CD79A, CD79B, CD37	25799053, 25254006, 6970772, 16181617, 12360049, 1592393, 2365993, 29230012, 29361178, CD Handbook
4	CD8A, GZMK	CD8+ T Cell	0.93	CD8A	28263960, 10754519, 10640724, 29230012, 29361178

Showing 1 to 5 of 9 entries Previous 1 2 Next


B Cell
CD14+ Monocyte
Non-Classical Monocyte
Plasmacytoid Dendritic Cell
Megakaryocyte, Platelet
T Cell
CD8+ T Cell
Naive T Cell
Regulatory T Cell
UMAP_1
UMAP_2
CD8A
Regulatory T Cell
B Cell
Non-Classical Monocyte
T Cell
Naive T Cell
CD8+ T Cell
CD14+ Monocyte
Plasmacytoid Dendritic Cell
Megakaryocyte, Platelet

CD8A
Expression Level
Regulatory T Cell
B Cell
Non-Classical Monocyte
T Cell
Naive T Cell
CD8+ T Cell
CD14+ Monocyte
Plasmacytoid Dendritic Cell
Megakaryocyte, Platelet
Identity