Automatic cell type annotation

≔ 相關類別

Dr. Po-Jung Huang, Chang Gung University, Taiwan. e-mail: pjhuang@gap.cgu.edu.tw



https://reurl.cc/94yIMO

1. Overview of cell annotation workflow 2. Strategies for automatic cell annotation 2-1 Marker-based annotation approach 2-2 Reference-based annotation approach 2-3 Strengths & pitfalls of marker-based and reference-based annotation approaches 3. Reference-based annotation of scRNA-Seq (SingleR) 3-1 Cell type reference datasets (celldex package) 3-2 Schematic of SingleR 3-3 SingleR browser 4. Marker-based annotation of scRNA-Seq 4-1 ScType 4-2 scCATCH 5. ShinySC 5-1 Upload 5-1-1 Input format: 5-1-2 Supported organisms: 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 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 5-6. Automated cell-type annotation 5-6-1 ScType 5-6-2 SingleR 5-6-3

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.

NATURE PROTOCOLS/VOL 16/JUNE 2021/2749-2764/

▼ 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)

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



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

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

▼ PanglaoDB (https://panglaodb.se)

Pangto DB & Home Q Search S Datasets - > Tools - I Papers ? FAQ/Help i About

PanglaoDB is a database for the scientific community interested in exploration of single cell RNA sequencing experiments from mouse and human. We collect and integrate data from multiple studies and present them through a unified framework.

Usage examples

- Run a gene search for SOX2, PECAM1 or ACE2
- Browse the full list of samples
- · Explore the list of cell type markers for Schwann cells
- · Browse cell types of the mouse retin
- · Look at the expression of CRX in photoreceptor cells
- Find cell clusters where both PECAM1 and VCAM1 are expressed using a boolean search with the 'and' operator
- · Find quiescent neural stem cells using AND+NOT

How to cite

Oscar Franzén, Li-Ming Gan, Johan L M Björkegren, PanglaoDB: a web server for exploration of mouse and human single-cell RNA sequencing data, Database, Volume 2019, 2019, baz046, doi:10.1093/database/baz046

What is single cell RNA sequencing?

Adapted from the Wikipedia article on the topic: Single cell RNA sequencing examines the transcriptomes from individual cells with optimized next generation sequencing technologies, providing a higher resolution of gene expression and a better understanding of the function of an individual cell in the context of its microenvironment.

Ba	Database stat	istics
Ya	Mus musculus	Homo sapiens
Samples	1063	305
Tissues 🕢	184	74
Cells 🕢	4,459,768	1,126,580
Clusters 🕢	8,651	1,748

Dataset of the day

Take a closer look at the cellular composition of Calvaria, using a dataset which consists of 369 cells. Clustering of this dataset resulted in 1 cell clusters, containing among others, Chondrocytes.

News

21-05-2020 Ongoing work to move to new hosting.
30-01-2020 A corrupted MySQL table caused dysfunction in the
search function, the problem has now been fixed.
28-11-2019 We are looking for sponsors to host PanglaoDB. We have
modest requirements (VPS with Ubuntu, etc). Please get in touch with
us if you can provide help (contact@panglaodb.se).
01-07-2019 Updated the 2d view for data sets (now colors by cell
type and not by cluster and colors are consistent across data sets). For
example, see this data set.
16–05–2019 Added more markers for Tanycytes.
07–05–2019 Added markers for Chromaffin cells.
01–05–2019 Markers for an additional cell types added: meet the
sebocyte.
a data da ser da ser de ser

-2019 Added sensitivity and specificity to the marker list (shown separately for mouse and human).

Show older news

▼ CellMarker (<u>http://117.50.127.228</u>)





▼ 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

NATURE PROTOCOLS|VOL 16|JUNE 2021|2749-2764|

▼ 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 <u>https://www.ncbi.nlm.nih.gov/geo/</u>)

GEO Home Documentation - Quer	y & Browse 👻 Email GEO		
Gene Expression Om GEO is a public functional genomics data repo sequence-based data are accepted. Tools are	nibus story supporting MIAME-compliant data submissions. Array- and provided to help users query and download experiments and curated		Gene Expression Ornelited
gene expression promes.			Keyword or GED Accession Search
Getting Started	Tools	Browse Conte	ent
Overview	Search for Studies at GEO DataSets	Repository Browse	er
FAQ	Search for Gene Expression at GEO Profiles	DataSets:	4348
About GEO DataSets	Search GEO Documentation	Series: 🔯	204017
About GEO Profiles	Analyze a Study with GEO2R	Platforms:	25181
About GEO2R Analysis	Studies with Genome Data Viewer Tracks	Samples:	6522606
How to Construct a Query	Programmatic Access		
How to Download Data	FTP Site		
	ENCODE Data Listings and Tracks		
Information for Submitters			
Login to Submit	Submission Guidelines	MIAME Standards	
	Update Guidelines	Citing and Linking	to GEO
		Guidelines for Rev	iewers
		GEO Publications	

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

Single	gle Cell Express cell gene expression across s entmetts & Cownoad % Release not	sion Atlas pocies s • • Help • Support			Query bulk expression	W
Search across 21 spec	cies, 355 studies, 10,505,726 cells		Ensem	bl 104, Ensembl Genomes	51, WormBase ParaSite 15, El	FO 3.10.0
Search						
11				Species		-
Animals Plants	Fungi Profisis					
1	1	X		1		
Homo sapi	ens Mus musculus	Drosophila melanogaster	Danio rerio	Gallus gallus	Rattus norvegicus	•
147 experim	ents 123 experiments	30 experiments	14 experiments	4 experiments	3 experiments	

- ▼ 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/

- 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: <u>https://www.mousecellatlas.org/</u>
- 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: <u>https://www.proteinatlas.org/</u>
- 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: <u>https://tabula-muris.ds.czbiohub.org/</u>
- 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: <u>https://www.flycellatlas.org/</u>

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

NATURE PROTOCOLS/VOL 16/JUNE 2021/2749-2764/

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 <u>known markers</u>	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 <u>novel cell types</u> or <u>subtypes</u> based on similarity to reference data	Requires high-quality and relevant annotated reference data
	Can be <u>more accurate</u> than marker-based methods for <u>well-matched</u> reference data	May perform poorly with <u>incomplete</u> or <u>poorly matched reference</u> <u>data</u> , which may result in <u>conflicting or absent cell labels</u>
	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

ChatPDF - Chat with any PDF!

ChatPDF is the fast and easy way to chat with any PDF, free and without sign-in. Talk to books, research papers, manuals, essays, legal contracts, whatever you have! The intelligence revolution is here, ChatGPT was just the beginning!

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. <u>doi:10.1038/s41590-018-0276-y</u>.

▼ 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)

MonacolmmuneData

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

NovershternHematopoieticData

Novershtern Hematopoietic Cell Data - GSE24759

3-2 Schematic of SingleR

Mouse (2)

ImmGenData

the murine ImmGen (Heng et al. 2008)

MouseRNAseqData

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



▼ Step1: Identifying variable genes

	-	-	-				veidan xpres:	sion	
	cell1	cell2	cell3	cell4	cell5	1	âlues O		0
gene1	*	*	*	*	*	gene1	*	*	*
gener	*	*	*	*	*	⇒gene2	*	*	*
genez	*	4	+	4	*	gene3	*	*	*
gene3	ф.	್	· T	·Ψ.	T.	0,000		16/2/07	
					• • •				
	*	s the median o	of the group o	***					

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



 Differential expression between <u>each other cell type</u> and the <u>'red' cell type</u> was

calculated and all genes with <u>positive differential</u> expression values were

selected

• The top *N* genes that showed the most difference in expression were chosen for the "red" cell type as <u>variable genes</u>.

▼ 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

inal	a browcar									
ingle	eR browser									
C	incle	• •••	ingle of		unition of call	+				
21	ingle	U ²	<u>ingie</u> -ce	n <u>H</u> ecog	Inition of cell	types				
Data	a sets Analysi	is Cluster	Proportions	Differential anal	ysis					
mport	tant note: the Sing	geR browseR ha	as been upgraded	d. It now uses a ne	w SingleR object. Please re	fer to https://	/github.	com/dviraran/SingleR for	more information.	
To ana	alyze single-cell da	ta sets choose a	an item from the ta	able and/or upload	SingleR objects.					
mpor	rt Data (SingleR ol	bject)								
Dem	No file on	and a second								
Brov	wse No file se	elected								
Brov	wse No file se	alected		1 al da an an 18 an 1						
Brov f you	wse No file se would like to add a	elected a dataset to this	list please contac	t dvir.aran@ucsf.ee	du.				Search:	
Brov f you t Show	wse No file se would like to add a 10 🕑 entrie Set.Name	a dataset to this as Organism	list please contac Citation	t dvir.aran@ucsf.ec Technology I	du. SingleR.Refs	N.cel	ls -	Title	Search:	PMI
Brow f you t Show	would like to add a 10 C entrie Set.Name GSE74923	a dataset to this as Organism Mouse	list please contact Citation	tt dvir.aran@ucsf.ec Technology 0 C1	du. SingleR.Refs Immgen, Mouse-RNAseq	0 N.cel	ls	Title microfluidic platform enab NA-seq of multigeneration	Search:	9 PMI 2673
Brow f you t Show 1	wse No file se would like to add a 10 C entrie Set.Name GSE74923 GSE78779	a dataset to this organism Mouse Mouse	list please contact Citation Kimmerling et al 2016 Hashimshony et al. 2016	Technology © C1 CEL-Seq2	du. SingleR.Refs Immgen, Mouse-RNAseq Immgen, Mouse-RNAseq	• N.cel	ls A 189 R 188 C	Title microfluidic platform enab NA-seq of multigeneration EL-Seq2: sensitive highly- ell RNA-Seq	Search:	 PMI 2673: 2712
Brow f you 55how 1 2 3	wse No file se would like to add a 10	a dataset to this organism (*) Mouse Mouse	list please contact Citation Kimmerling et al 2016 Hashimshony et al. 2016 Shalek et al. 2014	Technology © C1 CEL-Seq2 Drop-seq	du. SingleR.Refs Immgen, Mouse-RNAseq Immgen, Mouse-RNAseq Immgen, Mouse-RNAseq	N.cel	Is A 189 R 188 C 188 C 0 311 S	Title microfluidic platform enab NA-seq of multigeneration EL-Seq2: sensitive highly- ell RNA-Seq ell RNA-seq ingle-cell RNA-seq reveals ontrol of cellular variation	Search: bling single cell tal lineages multiplexed single- s dynamic paracrine	 PMI 2673; 2712; 24919;

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 oreate 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')

Abbreviated cell types OPC ASC OLG OEC Nent (. Milt11 expressed at varying levels across clusters DC MNC HypE NEU S100all Ctsd Ctsd Atp5g1 H2at2 Mbp Ptn Parci1 Aldoc Glat Ddah1 Ddah1 Don Spp2 Gm12144 Hbb-bs Hba-a2 Rarres2 Cd74 sel alla Ato 1 Men . 50% 25% ¢1 S3304 TAPE -Marker genes (] = clear marker genes



4. Marker-based annotation of scRNA-Seq

NATURE PROTOCOLS/VOL 16/JUNE 2021/2749-2764/



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 (<u>https://sctype.app</u>)**, and as **an open-source Rpackage (**<u>https://github.com/lanevskiAleksandr/sc-type</u>).

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)**.

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

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

	species	tissue	cancer	condition	subtype1	subtype2	s
8	Human	Periosteum	Normal	Normal cell	Periosteum- Derived	NA	r
9	Human	Periosteum	Normal	Normal cell	Periosteum- Derived	NA	r
10	Human	Periosteum	Normal	Normal cell	Periosteum- Derived	NA	r
11	Human	Amniotic membrane	Normal	Normal cell	Amnion	NA	٢
12	Human	Amniotic membrane	Normal	Normal cell	Amnion	NA	٢
13	Human	Primitive streak	Normal	Normal cell	Primitive	NA	٢
14	Human	Primitive streak	Normal	Normal cell	Primitive	NA	٢
15	Human	Adipose tissue	Normal	Normal cell	NA	NA	٢
16	Human	Scalp	Normal	Normal cell	Bulge	NA	٢
17	Human	Heart	Normal	Normal cell	NA	NA	٢
18	Human	Liver	Normal	Normal cell	NA	NA	٢
19	Human	Liver	Normal	Normal cell	NA	NA	٢



Clusters Matching & scoring 3 Clus в Step 2: construct CellMatch reference database Markers ⊖-Species ⊖-Tissue ⊖-Cell ⊖-Marker 00 Cell types

D

Markers genes

Step 1: identify cluster potential marker genes



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

obj <- findmarkergene(object = obj,</pre> 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"), ]</pre>
obj <- findmarkergene(object = obj, if_use_custom_marker = TRUE, marker = cellmatch_new)</pre>
obj <- findcelltype(obj)</pre>
# 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)</pre>
obj <- findcelltype(obj)</pre>
# Example
cellmatch_new <- cellmatch[cellmatch$species == "Mouse", ]</pre>
cellmatch_new <- cellmatch[cellmatch$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)</pre>
obj <- findcelltype(obj)</pre>
```

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

Uity control Ingut data format: 10% Genomics (Lip) Seurat R object (.rds) BD Rhapsody (.csv) CellView (.Rds): derring. Choose organism: (muckers. Human type zanoczatori Choose a file or drag It here.										
Imakes Muman ype annotation type annotation thoose a file or drag it here.										
type annotation Choose a file or drag it here.										
1 pbmc.rds										
Updated complete	tipbad comparie									
pbmc.uds has been successfully uploaded.	-									
Example 3 10X Genomics (Example 3) Seural Robject (Example 3) BD Rhapsody	Suample 4 CellView									
Download Deveload Dupper of the cell ranger pipeline from 1DX Securit R object [r/d] Output from BD Rhapsody Single-Cell Analysis	Lownload Dutput from CellView (. Rds format)									
Consuming (rink)										

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
```







5-3-2 Define optimal resolution

► STEP 4 (Clustering)	-	How many clusters to use?
Select Number of PCs:		A tree of clusters!
10	8	1. Cluster at multiple resolutions
Choose optimal resolution:		3. Build a graph edge samples
0.5	٢	5. Visualise In prop = high as sample
		Structure can
		3 Show stability
		5 Show information across resolutions

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

<u>Biomart:</u> Human (ok), Mouse (待確認Gene name格式)

🤉 New 📑 Count 📰 Results			*	JRL 🖻 XML 🖉 Peri 🕐 Help			
Dataset 43501 / 69299 Genes	Export all r	esults to	File	🥫 τεν 🥫 🕑 Unique results only 🧔	Go		
Human genes (GRCh38.p13)	Email notific	ation to					
Filters							
[None selected] With GeneCards ID(s): Only	View	6	200 👩 TOWS AS HTML	Unique results only	X).		
Attributes	Gene name	Gene description			GeneCards ID		
Conspons	MT-TE	mitochondrially encoded tRNA-Phe (UUU/C) [Source:HGNC Symbol:Acc:HGNC:7481]					
Gene name	MT-BNB1	mitochondrially encoded 12S rRNA [S	ource:HGNC Symbol;Acc:H	(GNC:7470)	7470		
Gene description	MT-TV	mitochondrially encoded tRNA-Val (GI	UN) [Source:HGNC Symbo	Acc:HGNC:7500]	7500		
GeneCards ID	MT-RNR2	mitochondrially encoded 16S rRNA [S	ource:HGNC Symbol;Acc:+	IGNC:7471]	7471		
	MT-TL1	mitochondrially encoded tRNA-Leu (U	UA/G) 1 [Source:HGNC Sy	mbol;Acc:HGNC:7490)	7490		
	MT-ND1	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 1 [Source:HGNC Symbol;Acc:HGNC:7455]					
ataset	MT-TI	mitochondrially encoded tRNA-lie (AU	U/C) [Source:HGNC Symb	ol;Acc:HGNC:7488]	7488		
Ione Selected]	MT-TQ	mitochondrially encoded (RNA-Gin (CAA/G) [Source:HGNC Symbol;Acc:HGNC:7495]					
	MT-TM	mitochondrially encoded tRNA-Met (AUA/G) [Source:HGNC Symbol;Acc:HGNC:7492]					
	MT-ND2	mitochondrially encoded NADH:ubiqui	inone oxidoreductase core	subunit 2 [Source:HGNC Symbol;Acc:HGNC:7456]	7456		
	WT-TM	mitochondrially encoded tRNA-Trp (U0	GA/G) [Source:HGNC Sym	bol;Acc:HGNC:7501]	7501		
	MT-TA	mitochondrially encoded tRNA-Ala (G0	CN) [Source:HGNC Symbo	(Acc:HGNC:7475]	7475		
	MT-TN	mitochondrially encoded IRNA-Asn (A	AU/C) [Source:HGNC Sym	bol;Acc:HGNC:7493]	7493		
	MT-TC	mitochondrially encoded tRNA-Cys (U	IGU/C) [Source:HGNC Sym	bol:Acc:HGNC:7477]	7477		
	MT-TY	mitochondrially encoded tRNA-Tyr (UA	AU/C) [Source:HGNC Symt	ool;Acc:HGNC:7502]	7502		
	MT-CO1	mitochondrially encoded cytochrome of	coxidase I [Source:HGNC :	Symbol;Acc:HGNC:7419]	7419		
	MT-TS1	mitochondrially encoded tRNA-Ser (U	CN) 1 [Source:HGNC Syma	tol;Acc:HGNC:7497]	7497		
			10 00 00				

▼ GeneCards

(GeneCardsID → GeneCards web site 連結待建立)

(GeneC	ards®		Free for academic no	n-profit institutions.Othe	r users need a <u>Co</u>	ommercial license	WEIZMANN INSTITUTE OF SCIENCE	🚷 LifeMap
	THE HUMAN GEN	NE DATABASE		Search Ger	neCards (supports bo	olean, parenth	esis and quotes)		Q Advanced
Home	User Guide Ar	nalysis Tools -	Release Note	s About - Data	Access GeneCa	rds Team		My Genes	Log In / Sign Up
VDA Pseudo	C1P4 Ger	n e - Volta ar 21, 2023 ; G	ge Depende co1P180434 @ ;	ent Anion Chanr GIFIS: 10 🛛 😚 🚔	nel 1 Pseudog	ene 4			Search in Gene Q Follow Gene ★ 🕸
Jump to section	Aliases Paralogs	Disorders Pathways	Domains Products	Drugs Proteins	Expression Publications	Function Sources	Genomics Summaries	Localization Transcripts	n Orthologs s Variants
Research Products	Antibodies Primers	Assays	Proteins	Inhib. RNA	CRISPR	miRNA	Drugs	Cell Lines	Clones
RD	Proteins Primary ELISAs Antibody Activity Assays	/ Antibodies / Arrays	(§) VectorBuilder	Online Vector Design Platform Virus Packaging (AAV/Lenti) CBISDD Lineau Construction		Proteins Antibo Genes shRNA F CRISPR Lentivir	dies Assays Primera ral Particles	SYNTHEGO	IISPR Knockout Kit sgRNA gineered Cells Ited IPSCs
Aliases fo GeneCai Voltage Voltage- VDAC4P VDAC4 ³ Voltage- External lo HGNC: 1	tor VDAC1P4 Gene rVDAC1P4 Gene rds Symbol: VDAC1 Dependent Anion Ch 35 Dependent Anion Ch ds for VDAC1P4 Gen 2675 NCBI Entre Co GNC Symbols for V	PP4 2 Channel 1 Pseud channel 4 Pseud channel 4 2 e Sene: 7418 En:	udogene 4 ^{2 3 5} logene ^{2 3} sembl: ENSG0000	0235060 OMIM®: 6100	30			Gener	ArDs BATCH QUERIES 190 Integrated Biomedical Sources APL JSON
VDAC4, Previous (VDAC4P SeneCards Identifier	rs for VDAC1P	4 Gene						CSV, EXCEL

Search aliases for VDAC1P4 gene in PubMed and other databases

1.Upload	Differential e	xpression tes	st: 🚺	c	luster:	0	avg_logFC>=		p_val_adj <	
2. Quality control	Wilcoxon Ra	nk Sum test	•		0		0.25		0.05	
3.Feature selection	Cluster-sp	ecific gene	marker(s)							- 2
5.Find markers	Сору	CSV E	cel PDF	Print					Search:	
6.Cell type annotation	gene	cluster	avg_log2FC	pct.1	pct.2	p_val_adj	p_val	description 🔞		GeneCardsID
	CCR7		1.389	0.468			4.29207278967683e-92	C-C motif chemokine recep Symbol;Acc:HGNC:1608]	itor 7 (Source: HGNC	160
	LDLRAP1	D	1.176	0.266	0.084	1.05255590972489e-30	3.21508922269195e-35	low density lipoprotein rec Symbol;Acc:HGNC:18640]	eptor adaptor protein 1 (Source:HGN	C 1854
	LEF1	0	1.092	0.358	0.105	1.74637802299883e-49	5.33440657034282e-54	lymphoid enhancer bindin Symbol;Acc:HGNC:6551]	g factor 1 [Source:HGNC	655
	PRKCQ- A51	0	1.082	0.356	0.109	2.26908277300734e-46	6.93103663329264e-51	PRKCQ antisense RNA 1 (Sc	aurce:HGNC Symbol;Acc:HGNC:44685) 4465
	MAL	0	1.020	0.29	0.085	7.83275525166572e-37	2.39255765522198e-41	mal, T cell differentiation p Symbol;Acc:HGNC:6817]	rotein [Source:HGNC	681
	LDHB	0	1.020	0.924	0.598	2.38977511626939e-100	7.29969795427147e-105	lactate dehydrogenase B [S	Source:HGNC Symbol;Acc:HGNC:6543] 654
	РІКЗІР1	0	1.005	0.467	0.183	2.14836457136785e-45	6.56229632649476e-50	phosphoinositide-3-kinase Symbol;Acc:HGNC:24942]	interacting protein 1 [Source:HGNC	2494
	NOSIP	0	0.975	0.657	0,358	2.21001609005487e-47	6.75061424049995e-52	nitric oxide synthase intera Symbol;Acc:HGNC:17946]	icting protein [Source:HGNC	1794
	CD3D	0	0.902	0.85	0.417	5.17841746277598e-66	1.58177575379558e-70	CD3 delta subunit of T-cell Symbol;Acc:HGNC:1673]	receptor complex (Source:HGNC	167
	CD3E	0	0.844	0.736	0.408	1.61002580496134e-45	4.91791131089065e-50	CD3 epsilon subunit of T-ce Symbol;Acc:HGNC:1674]	ell receptor complex [Source:HGNC	167
	Showing 1	to 10 of 166 e	ntries					Dentinue		177 10.00

Identification of Cluster-specific gene markers

.tuster-sp	ecinc gene marke	a(s)					2
Сору	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	Contraction (4.29207278957683e-92	C-C motif chemokine receptor 7 [Source:HGNC Symbol;Acc:HGNC:1608]
LDLRAP1		1.176	0.266	0.084		3.21508922269195e-35	low density lipoprotein receptor adaptor protein 1 [Source:HGN0 Symbol;Acc:HGNC:18640]
LEFI		1.092	0.358	0.105		5.33440657034282e-54	lymphoid enhancer binding factor 1 [Source:HGNC Symbol;Acc:HGNC:6551]
PRKCQ- AS1		1.082	0.356	0.109		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]
howing 1 t	to 10 of 166 entries					Prev	vious 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 (<u>https://sctype.app</u>), and as <u>an open-source R-package</u>.

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





5-6-2 SingleR

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

Dvir Aran, Agnieszka P. Looney, Legian 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



singler - Default

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

▼ singler web application

Single Single -cell Recognition of cell types

Data sets Analysis Cluster Proportions Differential analysis

Important note: the SingeR browseR has been upgraded. It now uses a new SingleR object. Please refer to https://github.com/dviraran/SingleR for more information.

Inport data induit the singler blowser mes been opgraded, it now uses a new singler object. To analyze single cell data sets choose an item from the table and/or upload SingleR objects. Import Data (SingleR object) Browse... No the selected

If you would like to add a dataset to this list please contact dvir.aran@ucsf.edu. Show 10 Bentries

	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	2673228
2	GSE76779	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 (Zhong) - 2000cells	Human	Zheng et al. 2017	10X	HPCA, Blueprint_Encode	4099	Massively parallel digital transcriptional profiling of single cells.	28091601
5	GSE111664	Mouse	Aran et al.	Drop-seq	Immgen, GSE94135+GSE49932, Mouse-RNAseq	8405	Single-cell RNA-seq reveals profibrotic macrophages in lung fibrosis	NA
6	PBMC (healthy)	Human	10X datasets	10X	HPCA, Blueprint_Encode	8391	Massively parallel digital transcriptional profiling of single cells,	28091601
7	TabulaMuris-FACS	Mouse	Tabula Muris	Smart-Seq2	immgen, Mouse-RNAseq	44949	Single-cell transcriptomics of 20 mouse organs creates aTabula Muris	NA
8	GSE52529	Human	Trapnell et al. 2014	C1	HPCA, Blueprint_Encode	379	Pseudo-temporal ordering of individual cells reveals regulators of differentiation	24658644
9	GSE54006	Mouse	Jaitin et al. 2014	MARS-Seq	Immgen, Mouse-RNAseq	1864	Massively parallel single-cell RNA-Seq for dissecting cell type and cell state compositions	24531970
10	GSE57872	Human	Patel et al. 2014	Smart-Seq	HPCA, Blueprint_Encode	96	Single cell RNA-seq of primary human glioblastomas	24925914

Reference Index for cell type **@**

biocmanager::install("celldex")

• 建立local databases

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

1.Upload	Cell-type identification methods
2.Quality control	🔿 ScType 🛞 SingleR 🔿 scCATCH
(파) 3.Feature selection	
◀ 4.Clustering	Reference dataset:
Q. S.Find markers	(Human) NovershternHematopoleticData
	(Human) RovershternHernatopoleticData: Innume Maniera; dataetis In surbit Servitapilied; all papabilism (Investidaet) at al. 2011
# 6.Cell type annotation	(Human) DatabaseImmuneCellExpressionData immuneBulk #WA-seq datasets from the DICE (Database of Immune Cell Depression, Depression quantitative trait loo (eQTLs) and Epigmonical project
	(Human) MonacolimmuneData ImmuneBulk 894-seg samples of sorted Immune cell populations-sit=(Monaco et al. 2019)
	(Human) HumanPrimaryCellAtlasData Generali Publicly available microarray datasets derived from human primary cells (Naobott et al. 2013)
	(Human) BlueprintEncodeData Generalifulk RNA-seq data for pure stroma and immune cells generated by Blaeprint-val-and ENCODE projects.







5-6-3

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

Xin Shao¹, Jie Liao¹, Xiaoyan Lu¹, Rui Xue¹, Ni Ai¹, Xiaohui Fan²

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

<u>main.pdf</u>

https://github.com/ZJUFanLab/scCATCH

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

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

shinySC	=											
 ▲ LUplowd ✓ 2.Quality control (=) 3.Feature selection 	Cell-type identification methods scrype Singlet ® scarce											
4 A.Costering	Select reference datasets for cell type annotation	Datasets deposit	ed in CellWatch	reference data	ódse							- 7
Q. 5 Find markers	Normal/Cancer:	species	cancer	timue	cellitype	gene .	pmid	resource	condition	subtype1 ·	subtype2	subtype3
🖉 6 Cell type annotation	Normal +	153 Human	Normal	Blood	T Cell	CD3D	26453337	Esperiment	Normal cell	Angiogenic		
	Tissue: Bloof	354 Human 355 Human 155 Human	Normal Normal	Blacd Blood Blacd	T Cell T Cell T Cell	CD3E CD3E RECAMI	26453327 26453327 26453327	Experiment Experiment	Normal cell Normal cell Normal cell	Angiogenic Angiogenic Angiogenic		
	Cell Inner	157 Human	Normal	Blood	T Cell	CXCR4	26453327	Espariment	Novinal cell	Angiogenic		
	It Cell, Bassphil, Ornalating Fetal Cell, Decidual Cell, Bendmic Cell, Indozbellal Ce 🗢	789 Humat	Normal	Blood	Dendritic Cell	AXL.	28428369	Single-cell sequencing	Normal cell	AXL+		
	Select method for marker genes identification	381 Human	Normal	filood	Monocyte	CD14	19001052	Experiment	Normal cell	CD14+		
	🔿 scCATCH (Slow) 🌻 Sevent (Past)	184 Human	Normal	Blood	Monocyte	CD14	19001052	Experiment	Normal cell	CD24+		
	IN REAL	99 Human 100 Human	Normal Normal	Blood	Progenitor Cell Progenitor Cell	I PROML I CD34	24055327 24055327	Experiment Experiment	Normal cell Normal cell	Circulating Circulating		

10101-0000000	
Normal	
Kaposi's Sarcoma	
Larynx Cancer	
Leukemia	
Lipoma	
Liver Cancer	
Lung Adenocarcinoma	
Lung Cancer	
Lung Squamous Cell Carcinoma	
Lymphoma	
Malignant Insulinoma	
Malignant Mesothelioma	
Malignant Peripheral Nerve Sheath Tumor	
Medulloblastoma	
Melanoma	
Mucoepidermoid Carcinoma	
Multiple Myeloma	
Myeloma	
Natural Killer Cell Lymphoma	
Nephroblastoma	
Non-Small Cell Lung Cancer	
Normal	
Oesophageal Cancer	
Oligodendroglioma	
Oral Cancer	
Oral Squamous Cell Carcinoma	
Osteosarcoma.	
Ovarian Cancer	
Ovarian Cancer Pancreatic Cancer	
Ovarian Cancer Paricreatic Cancer Paricreatic Ductal Adenocarcinomas	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Papillary Thyroid Carcinoma	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Papillary Thyroid Carcinoma Prostate Cancer	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Papillary Hyroid Carcinoma Postate Cancer Renal Cell Canchoma	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Papillary Hyroid Carcinoma Prostate Cancer Remai Cell Carcinoma Remai Cell Carcinoma	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Papillary Thyroid Carcinoma Prostate Cancer Renal Cell Carcinoma Retinoblastoma	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Postate Cancer Renal Cel Cancinoma Renal Clear Cell Carcinoma Renal Clear Cell Carcinoma Salivary Gland Tumor	
Ovarian Cancer Pancreatic Cancer Papillary Thyroid Carcinoma Papillary Thyroid Carcinoma Postate Cancer Renal Cell Carcinoma Renal Clear Cell Carcinoma Retinoblatoma Salivary Gland Tumor Sarcoma	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Papillary Thyroid Carcinoma Postate Cancer Renal Cell Carcinoma Renal Clear Cell Carcinoma Retinoblastoma Sallvary Gland Tumor Surroma Small Cell Lung Cancer	
Ovarian Cancer Pancreatic Cancer Pancreatic Ductal Adenocarcinomas Postata Cancer Remail Cell Carcinoma Renat Clear Cell Carcinoma Retinoblastoma Salivary Gland Tumor Sarcoma Sanial Cell ung Cancer Testicular Germ Cell Tumor	
Ovarian Cancer Pancreatic Cancer Papilitary Thyroid Carcinoma Positate Cancer Renal Cell Carcinoma Renal Cell Carcinoma Renal Cell Carcinoma Belinoblatoma Salivary Gland Tumor Sarcoma Small Cell Ing Cancer Tstisticular Germ Cell Tumor Thyroid Cancer	
Ovarian Cancer Pancreatic Cancer Papillary Thyroid Carcinomas Papillary Thyroid Carcinoma Postata Cancer Renat Cell Carcinoma Renat Cell Carcinoma Retinablastoma Salivary Gland Tumor Sarcoma Small Cell Lung Cancer Tasticular Germ Cell Tumor Tasticular Germ Cell Tumor Torgoid Cancer	



ell, Basophil, Circulating Fetal Cell, Decidu	al Cell, Dendritic Cell, Endothelial Ce
Select Al	Deselect All
celitype	
B Cell	
Basophil	
Circulating Fetal Cell	
Decidual Cell	
Dendritic Cell	
Endothelial Cell	
Eosinophil	
Epithelial Cell	
Erythroblast	
Granulocyte	
Hematopoietic Cell	
Killer Cell	
Leukocyte	
Lymphocyte	
Lymphoid Cell	
Macrophage	
Megakaryocyte	
Monocyte	
Myeloid Cell	
Neutrophil	
Plasmablast	
Platelet	
Progenitor Cell	
Progenitor-like Angiogenesis-promoting C	ell
Red Blood Cell (Erythrocyte)	
Stern Cell	
T Cell	
Thymic Emigrant Cell	
White Blood Cell	

• The differences between Seurat and scCATCH

S	elect reference datasets for cell type annotation —
N	ormal/Cancer:
	Normal
т	issue:
	Blood
с	ell type:
	B Cell, Basophil, Circulating Fetal Cell, Decidual Cell, Dendritic Cell, Endothelial Ce
S	elect method for marker genes identification
	scCATCH (Slow) 🔘 Seurat (Fast)

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

how 5	entries			Search:	
cluster	cluster_marker	cell_type	celltype_score	celltype_related_marker	PMID
ĺ.	JUN, SELL, CKCR4, IL7R, LTB, GIMAP7, RGS10, CD3E, CD3D, EVL, JUNB, DNAJB1, PIK3IP1	Angiogenic T Cell	0.92	CD3D, CD3E, CXCR4	264533
ŭ	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	264533
Ĺ	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, LY2, NPC2, MT2A, ARRB2, TYROBP, FTL, TYMP, ITGB2	Basophil	0.88	CD63	283429 261846 214218 205589 199456 188178 147074
	CD52, SELL, CXCR4, PLAC8, LTB, HLA-DPA1, HLA-DPB1, SNX3, PTPRCAP, CD69	T Cell	0.75	CXCR4, SELL, CD69	264533 226970 285663
	CD2, ID2, DUSP2, IL7R, HLA-E, CD99, ANXAL, CTSW, PTPRCAP, CD3E, CD3D	Angiogenic T Cell	0.91	CD3D, CD3E	264533





[▼] scCATCH approach

how 5	entries			Search:	
cluster	cluster_marker	cell_type	celltype_score celltype_related_marker	PMID	
)	CCR7	TCell	0.5 CCR7	28929596	
	CD2, ILTR, LTB, GIMAPT, CD3E, CD3D	Angiogenic T Cell	0.91 CD3D, CD3E	26453327	
	\$100A9, \$100A12, \$100A8, CD14, FCN1, ASGR1	CD14+ Monocyte	0.88 CD14	19001052	
	MS4A1, TCL1A, CD79B	B Cell	0.75 MS4AI	25254006, 6970772, CD Handbook	
6	CD8A	CD8+ T Cell	0.8 CD8A	10754519, 10640724	

Automatic cell type annotation





cluster	cluster_marker	cell_type	celltype_score	celltype_related_marker	PMID
	CCR7	Naive T Cell	0.85	CCR7	23044634, 29361178, 28622514
	CD2, IL7R, LTB, GIMAP7, CD3E, CD3D, LDHB	Regulatory T Cell	0.96	CD3D, CD3E, IL7R	28777444, 26049548, 23769051
	S100A9, S100A12, S100A8, CD14, FCN1, ASGR1	CD14+ Monocyte	0.94	CD14	19001052, 29361178
	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
(CD8A, GZMK	CD8+T Cell	0.93	CD8A	28263960, 10754519, 10640724, 29230012, 29361178



identity