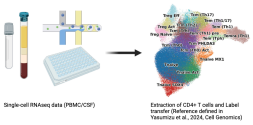
Mar 28, 2025

PBMC/CSF single-cell RNAseq CD4+ T cell reference mapping



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OPEN ACCESS

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External link: https://github.com/yyoshiaki/screfmapping

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Yasumizu, Yoshiaki, Daiki Takeuchi, Reo Morimoto, Yusuke Takeshima, Tatsusada Okuno, Makoto Kinoshita, Takayoshi Morita, et al. 2024. "Single-Cell Transcriptome Landscape of Circulating CD4+ T Cell Populations in Autoimmune Diseases." *Cell Genomics* 4 (2): 100473.

Protocol status: Working
We use this protocol and it's working

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Keywords: ASAPCRN, single-cell RNAseq, CD4+ T cells, PBMC, CSF, cell rnaseq cd4, cell populations in pbmc, detailed cluster assignment of cd4, cell genomic, pbmc, cell population, cd4, cell, detailed cluster assignment

Abstract

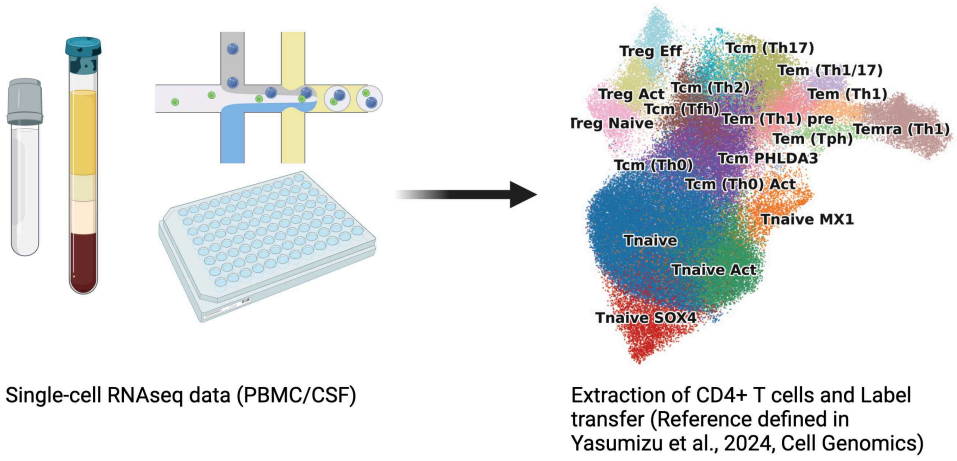
The pipeline for detailed cluster assignment of CD4+ T cells from PBMC or CSF. The pipeline utilize Azimuth and Symphony for the CD4+ T cell extraction and the label transfer. The pipeline was originally developed and used by Yasumizu et al., 2024, Cell Genomics. This pipeline has been used for various studies, contributing to identifying disease-associated T-cell populations in PBMCs and CSFs (Mori et al., Cell, 2024; Yata et al., Cell Genomics, 2025; Wei et al., JCI, 2025).

Materials

The pipeline is tested on Linux OS (Ubuntu, Red Hat) and not tested on MacOS or Windows.

Background

1



Single-cell RNAseq data (PBMC/CSF)

Extraction of CD4+ T cells and Label transfer (Reference defined in Yasumizu et al., 2024, Cell Genomics)

This pipeline will composed of two steps; 1. extraction of CD4+ T cells, 2. Label transfer from pre-defined CD4+ T cells.

Preparation

2 Prepare single-cell data. Whole PBMC/CSF data can be used. PBMC or CSF datasets generated by 10x platforms, Seq-Well, or SPLiT-seq (Parse Biosciences WT Mega) were tested. Another platform can also be used. Test data can be downloaded at https://cf.10xgenomics.com/samples/cell/pbmc3k/pbmc3k_filtered_gene_bc_matrices.tar.gz.

3 Clone the repository https://github.com/yyoshiaki/screfmapping

Bash

```
git clone https://github.com/yyoshiaki/screfmapping.git
```

4 A docker container can be used. Alternatively, a local environment can be used. We recommend Docker.

STEP CASE

Use Docker (Recommended) 5 steps

Alternatively, you can use singularity/Apptainer

5 Install Docker

6 Pull a docker image

bash

```
docker pull yyasumizu/screfmapping:0.0.1
```

Make a script

7 screfmapping/example.R can be used as the template. Update this example.R as follows.

1. Change parameters for the output. Updating project.name is fine.

example.R

```
## ----parameter setting (change here)----------------------------
----------------------------------
project.name <- "example"
prefix <- paste0("./output/", project.name, "/", project.name)
dir.create(paste0("./output/", project.name), recursive = T)
```

2. Load single-cell RNAseq object
In the example, the codes are importing raw 10x data. Instead, users can use their own separate objects. The Seurat object needs to include raw counts.

example.R

```
## ----reading data (change here)--------------------------------
-----------------------------------
# Load the PBMC dataset
pbmc.data <- Read10X(data.dir =
"/filtered_gene_bc_matrices/hg19/")
q <- CreateSeuratObject(counts = pbmc.data,
                        project = project.name,
                        assay = "RNA",
                        min.cells = 3,
                        min.features = 200)
```

Run the script

8 Run the script. Change /home/rstudio/autoimmune_10x to the working directory and example.R to the updated script.

bash

```
docker run --rm -it -v ${PWD}:/home/rstudio/autoimmune_10x
yyasumizu/screfmapping:0.0.1 Rscript example.R
```

Interpretation of the outputs

9 The two files are major outputs from the pipeline.

- ${prefix}_predict_clusterL1L2_Reference_Mapping.pdf
- ${prefix}_Reference_Mapping.csv : Assigned CD4+T clusters per cell.

example_Reference_Mapping.csv

```
,clusterL1,clusterL1_prob,clusterL2,clusterL2_prob
AAACATTGATCAGC-1,Tcm,1,TcmTfh,0.8
AAACGCACTGGTAC-1,Treg,1,TregEff,1
AAACGCTGTAGCCA-1,Temra,0.8,TemraTh1,0.8
AAACTTGATCCAGA-1,Tnaive,1,Tnaive,1
AAAGAGACGAGATA-1,Tcm,0.8,TcmTh0,0.6
AAAGAGACGGCATT-1,Tnaive,1,TnaiveMX1,0.8
AAAGCCTGTATGCG-1,Tem,1,TemTh1pre,1
AAAGTTTGTAGAGA-1,Tnaive,1,Tnaive,0.6
AAATCAACACCAGT-1,Tnaive,0.8,Tnaive,0.4
```

Protocol references

Reference

Yasumizu, Yoshiaki, Daiki Takeuchi, Reo Morimoto, Yusuke Takeshima, Tatsusada Okuno, Makoto Kinoshita, Takayoshi Morita, et al. 2024. “Single-Cell Transcriptome Landscape of Circulating CD4+ T Cell Populations in Autoimmune Diseases.” *Cell Genomics* 4 (2): 100473.

Code

https://github.com/yyoshiaki/screfmapping