

# HOW TO USE AZIMUTH TO DO CELL TYPE ANNOTATION

Xiaochen Zhang

MIG

0

Open Azimuth's official website:

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

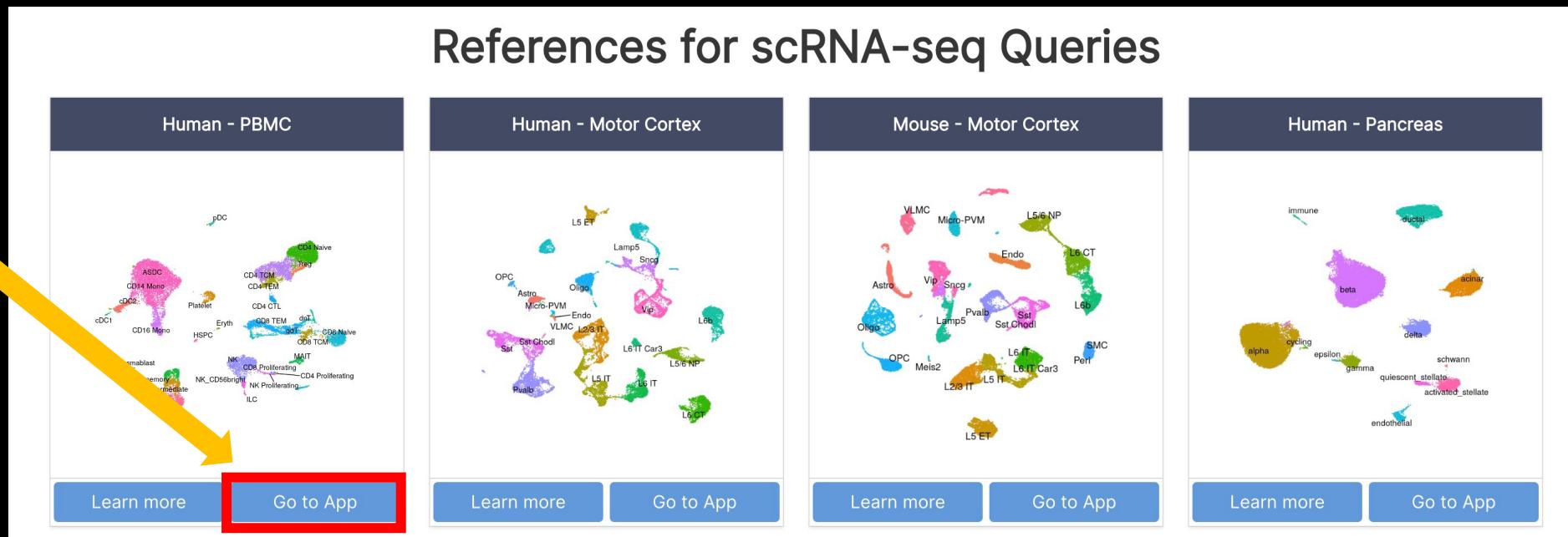
The screenshot shows the homepage of the Azimuth web application. At the top, there is a navigation bar with the HuBMAP logo on the left and 'Home' and 'References' links on the right. Below the navigation bar is a large banner featuring a vintage-style world map background. Overlaid on the map is the word 'Azimuth' in a large, white, serif font. Below 'Azimuth', the text 'App for reference-based single-cell analysis' is displayed in a smaller, white, sans-serif font. To the right of the banner is a detailed illustration of an antique compass rose. The main content area below the banner contains a paragraph of text describing Azimuth's purpose: 'Azimuth is a web application that uses an annotated reference dataset to automate the processing, analysis, and interpretation of a new single-cell RNA-seq or ATAC-seq experiment. Azimuth leverages a 'reference-based mapping' pipeline that inputs a counts matrix and performs normalization, visualization, cell annotation, and differential expression (biomarker discovery). All results can be explored within the app, and easily downloaded for additional downstream analysis.' Below this paragraph is another text block stating: 'The development of Azimuth is led by the New York Genome Center Mapping Component as part of the NIH Human Biomolecular Atlas Project (HuBMAP). Thirteen molecular reference maps are currently available, with more coming soon.' At the bottom of the page, there is a section titled 'References for scRNA-seq Queries' which displays four small t-SNE plots corresponding to different datasets: 'Human - PBMC', 'Human - Motor Cortex', 'Mouse - Motor Cortex', and 'Human - Pancreas'. Each plot shows clusters of colored points representing different cell types.

# 1

## Find ‘References for scRNA-seq Queries’

-> Then find ‘Human - PBMC’

-> click ‘Go to App’

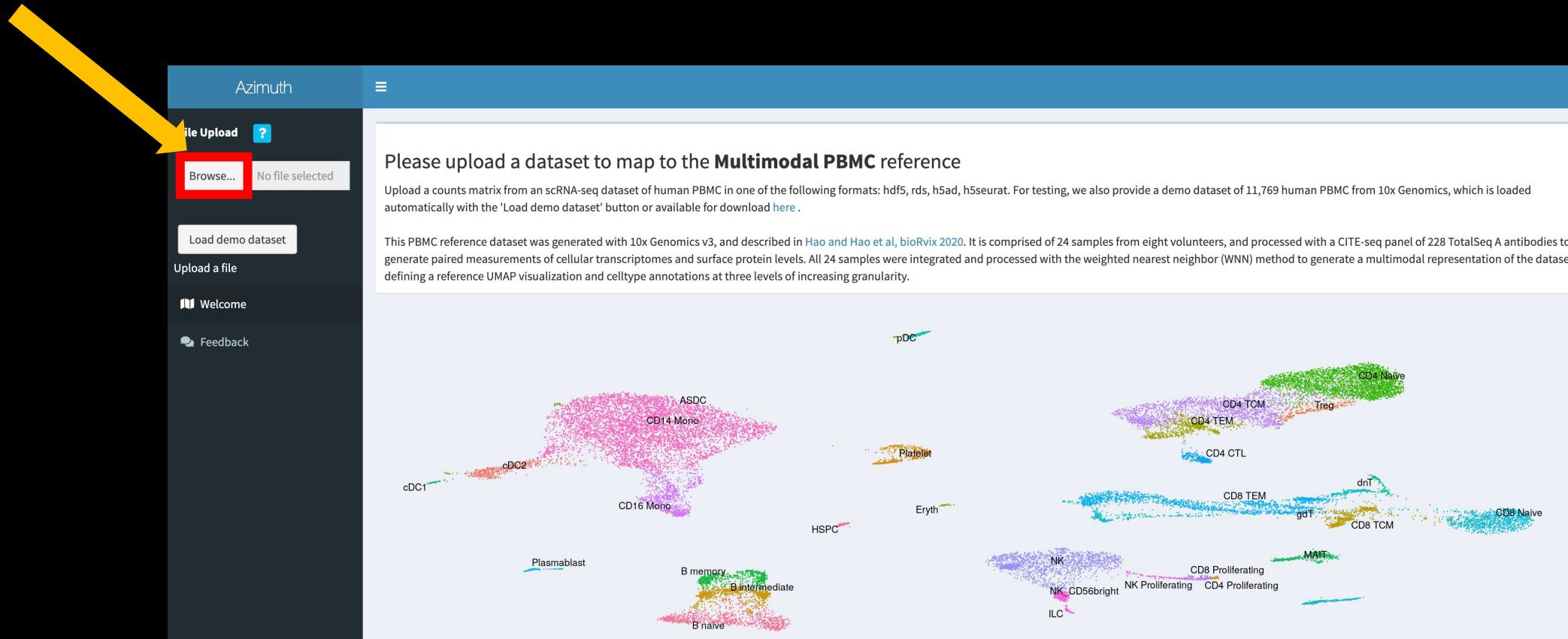


2

**Click 'Browse'**

-> find 'pbmc\_10x\_v2.Rds' at your working directory

—> Click 'Open'



# 3

## Waiting for the Rds file upload to the website

Azimuth

File Upload ?

Browse... pbmc\_10x\_v2.Rds  
pbmc\_10x\_v2.Rds

Load demo dataset

Upload a file

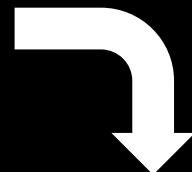
Welcome

Feedback

Please upload a dataset to map to the **Multimodal PB**

Upload a counts matrix from an scRNA-seq dataset of human PBMC in one of the following formats. You can upload your own dataset or load a demo dataset automatically with the 'Load demo dataset' button or available for download [here](#).

This PBMC reference dataset was generated with 10x Genomics v3, and described in [Hao and Regehr \(2018\)](#). It contains 24 samples of human PBMCs, each with paired measurements of cellular transcriptomes and surface protein levels. All 24 samples were used to define a reference UMAP visualization and celltype annotations at three levels of increasing complexity.



Azimuth

File Upload ?

Browse... pbmc\_10x\_v2.Rds  
Upload complete

Load demo dataset

3362 cells uploaded

Welcome

Preprocessing

Feedback

QC Filters ?

min nCount\_RNA 17 max nCount\_RNA 28015

min nFeature\_RNA 15 max nFeature\_RNA 4871

min percent.mt 0 max percent.mt 17

3362 cells remain after current filters

Transfer Options ?

Reference Metadata to Transfer celltype.l2

Map cells to reference

Log-scale Y-axis Hide points

nCount\_RNA nFeature\_RNA

0% 25% 50% 75% 100%

NUMI per cell 17.00 2024.25 2627.00 3357.25 28015.00

Genes detected per cell 15.00 905.00 1109.50 1281.00 4871.00

Mitochondrial percentage per cell 0.00 2.75 3.54 4.46 16.02

Sometimes errors occur due to bad network:

Try again or Use the tsv results we provide

## 4

Click 'Map cells to reference'

Azimuth

File Upload ?

Browse... pbmc\_10x\_v2.RData  
Upload complete

Load demo dataset  
3362 cells uploaded

Welcome

processing

Feedback

QC Filters ?

Log-scale Y-axis  Hide points

min nCount\_RNA max nCount\_RNA  
17 28015

min nFeature\_RNA max nFeature\_RNA  
15 4871

min percent.mt max percent.mt  
0 17

3362 cells remain after current filters

Transfer Options ?

Reference Metadata to Transfer  
celltype.l2

Map cells to reference

nCount\_RNA nFeature\_RNA percent.mt

query

0% 25% 50% 75% 100%

nUMI per cell	17.00	2024.25	2627.00	3357.25	28015.00
Genes detected per cell	15.00	905.00	1109.50	1281.00	4871.00
Mitochondrial percentage per cell	0.00	2.75	3.54	4.46	16.02

## 5

## Click 'Download Results'

Azimuth

File Upload ?  
Browse... pbmc\_10x\_v2.R  
Upload complete

Load demo dataset

3362 cells uploaded  
3362 cells preprocessed  
3362 cells mapped  
in 33 seconds

Welcome

Preprocessing

Cell Plots

Feature Plots

Download Results

Feedback

debug ID: 9ce5c172fb77  
Azimuth version: 0.3.2  
Seurat version: 4.0.0  
Reference version: 1.0.0

QC Filters ?

min nCount\_RNA: 17 | max nCount\_RNA: 28015  
min nFeature\_RNA: 15 | max nFeature\_RNA: 4871  
min percent.mt: 0 | max percent.mt: 17

3362 cells remain after current filters

Transfer Options ?

Reference Metadata to Transfer: celltype.l2

Map cells to reference

Log-scale Y-axis  Hide points

nCount\_RNA

nFeature\_RNA

percent.mt

0% 25% 50% 75% 100%

	0%	25%	50%	75%	100%
nUMI per cell	17.00	2024.25	2627.00	3357.25	28015.00
Genes detected per cell	15.00	905.00	1109.50	1281.00	4871.00
Mitochondrial percentage per cell	0.00	2.75	3.54	4.46	16.02

3362 cells uploaded ✓

3362 cells after filtering ✓

57.91% % of query cells with anchors ✓

5/5 cluster preservation score ✓

## 6

# Find 'Predicted cell types and scores (TSV)'

The screenshot shows the Azimuth web application interface. On the left, a sidebar contains navigation links: Welcome, Preprocessing, Cell Plots, Feature Plots, Download Results, and Feedback. The main area displays analysis results for a dataset uploaded from pbmc\_10x\_v2.Rds. The results include:

- Analysis script template:** A download button.
- UMAP (Seurat Reduction RDS):** A code block:

```
projected.umap <- readRDS('azimuth_umap.Rds')
object <- object[, Cells(projected.umap)]
object[['umap.proj']] <- projected.umap
```

A download button.
- Imputed protein (Seurat Assay RDS):** A code block:

```
imputed.assay <- readRDS('azimuth_impADT.Rds')
object <- object[, Cells(imputed.assay)]
object[['impADT']] <- imputed.assay
```

A download button.
- Predicted cell types and scores (TSV):** A code block:

```
predictions <- read.delim('azimuth_pred.tsv', row.names = 1)
object <- AddMetaData(
  object = object,
  metadata = predictions)
```

A download button.

A red box highlights the "Predicted cell types and scores (TSV)" section.

## 7

## Click 'Download'

The screenshot shows the Azimuth web application interface. On the left, a sidebar menu includes 'File Upload' (with 'pbmc\_10x\_v2.Rds' uploaded), 'Load demo dataset', and various plotting tools like 'Welcome', 'Preprocessing', 'Cell Plots', 'Feature Plots', 'Download Results', and 'Feedback'. The main content area displays several analysis results:

- Analysis script template:** A button labeled 'Download'.
- UMAP (Seurat Reduction RDS):** A code block:

```
projected.umap <- readRDS('azimuth_umap.Rds')
object <- object[, Cells(projected.umap)]
object[['umap.proj']] <- projected.umap
```

A yellow arrow points from this section towards the 'Download' button in the 'Predicted cell types and scores (TSV)' section.
- Imputed protein (Seurat Assay RDS):** A code block:

```
imputed.assay <- readRDS('azimuth_impADT.Rds')
object <- object[, Cells(imputed.assay)]
object[['impADT']] <- imputed.assay
```

A yellow arrow points from this section towards the 'Download' button in the 'Predicted cell types and scores (TSV)' section.
- Predicted cell types and scores (TSV):** A code block:

```
predictions <- read.delim('azimuth_pred.tsv', row.names = 1)
object <- AddMetaData(
  object = object,
  metadata = predictions)
```

This section has a red box around its 'Download' button.

8

Copy the tsv file (azimuth\_pred.tsv) to your R working directory

Finish!