



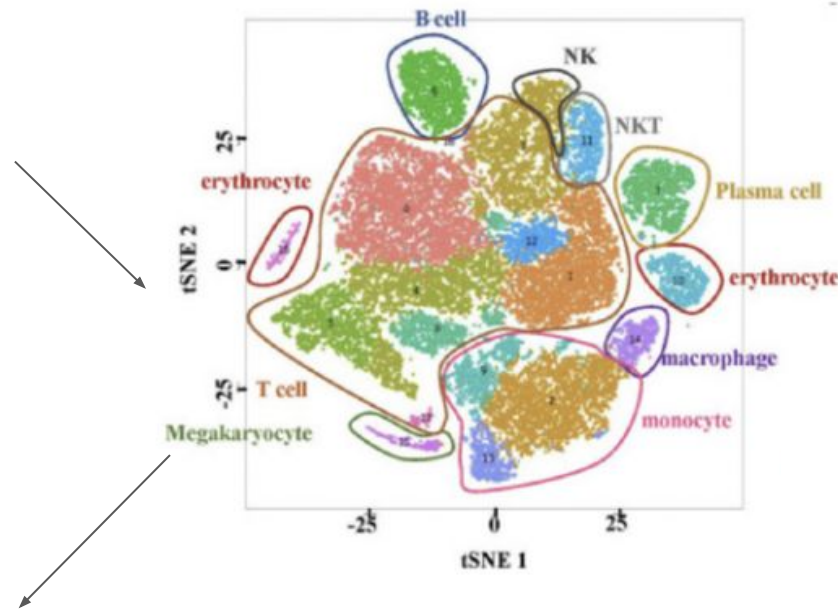
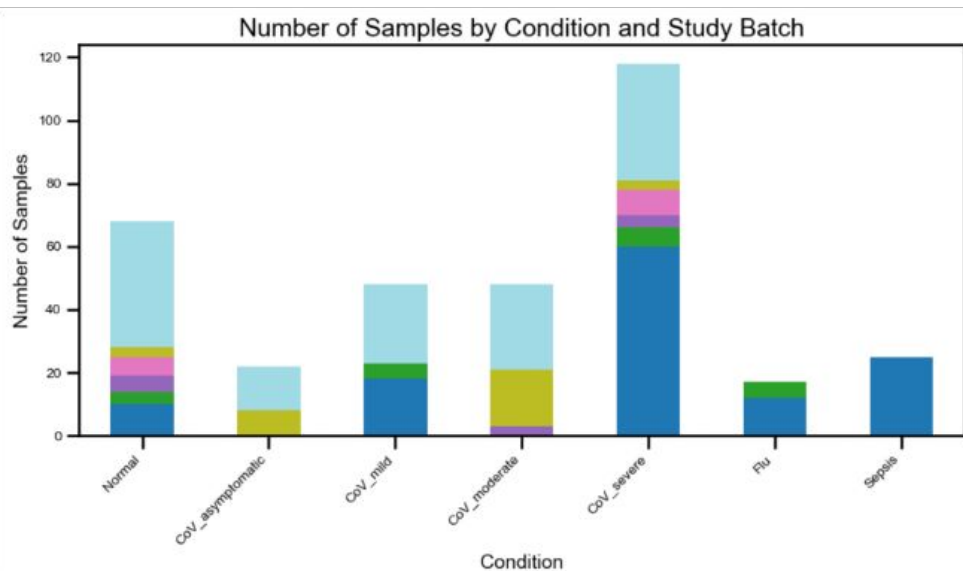
# HIPC scRNA-seq Annotation Benchmark Challenge

1st Webinar: April 28, 2026

HIPC Coordinating Center

# Reproducible and consistent cell annotation is the basis for scRNA-seq analysis

HIPC (Human Immunology Project Consortium): Pooling infection and vaccination resources for cell-resolution understanding under more broader contexts.



Reliability and reproducibility from such meta-analysis?

## HIPC scRNA-seq Annotation Benchmark Challenge

Introduction -  
Project  
Background

Dataset description,  
access and  
annotation templates

Submission  
walkthrough and  
Evaluation  
framework

Timeline and  
Summary

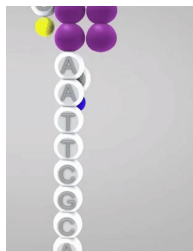
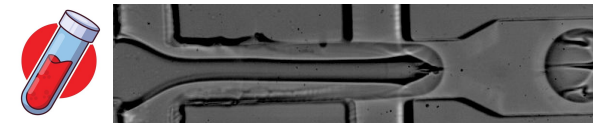
Siddharth Gaywala

Pramod Shinde,  
Jian Xing

Chuan He,  
Siddharth Gaywala

Pramod Shinde

# Immune cell-type annotation: Background



agg-TCR	7	.	7	9	33	8	.	12	29	.
agg-BCR	.	.	.	.	.	.	.	.	.	.
PF4	.	.	.	.	.	.	.	.	.	.
CD14	.	2	.	1	.	.	11	.	.	7
HBB	.	.	.	.	.	.	.	.	.	.
MT-CO2	44	56	16	51	58	21	21	38	46	89
SAMD11	.	.	.	.	.	.	.	.	.	.
NOC2L	.	.	.	.	1	.	1	.	.	.
KLHL17	.	.	.	.	.	.	.	.	.	.
PLEKHN1	.	.	.	.	.	.	.	.	.	.
PERM1	.	.	.	.	.	.	.	.	.	.

- **Definition:**

- labeling immune cell-types is critical for understanding immune responses in infection, vaccination, cancer, etc.

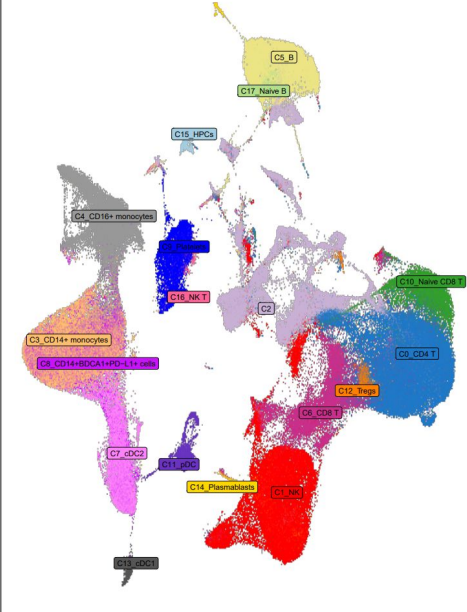
- **Biological challenges:**

- noise + sparsity in scRNAseq make it challenging to cutoff cells by a single gene
- lack of known, well-expressed marker genes in scRNAseq level for many subsets
  - many known cell surface markers not necessarily well expressed at RNA level
- clustering and then labeling clusters has issues bc cells cluster by TCR, cell-cycle, donor, etc.
  - cells can exist in continuous states too (naive → effector → memory; resting → activated → exhausted)

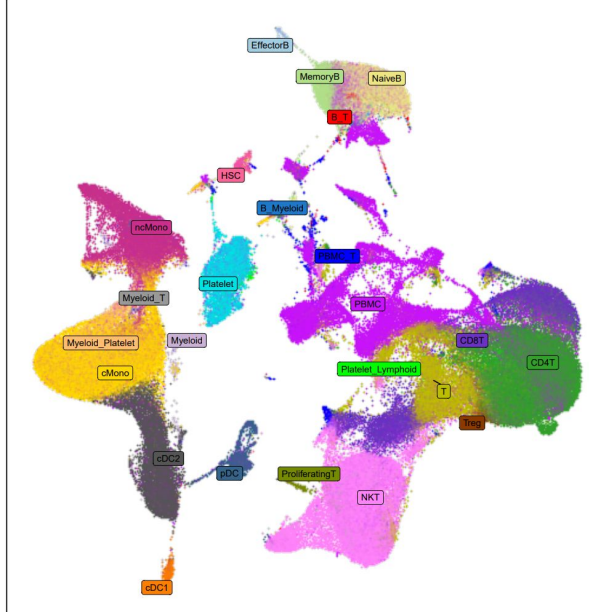
# Immune cell-type annotation - Analysis & Workflow Challenges

- no universal "ground truth" for many datasets
  - many annotations are manual: subjective, labor-intensive, poor reproducibility
- many automated tools don't give great depth or are sometimes very wrong
  - also often hard to audit why a specific label was assigned

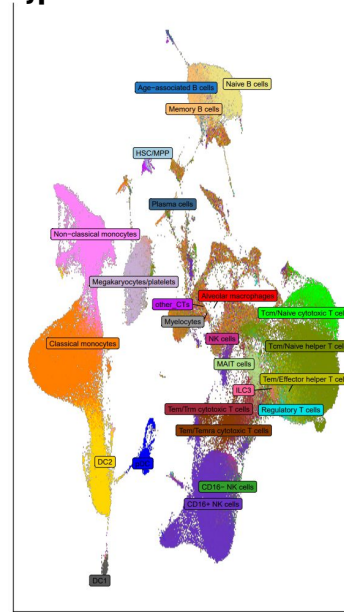
**Author Manual Annotation**



**2-Round Method Annotation**

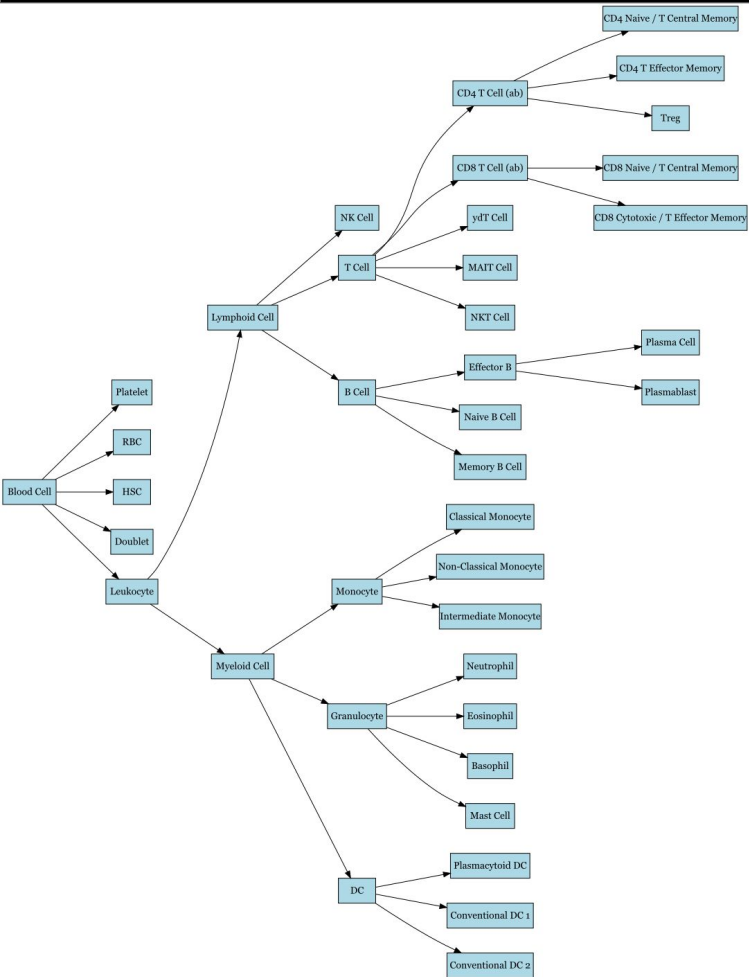


**Cell Typist Tool Annotation**



**Goal:** Evaluate the consistency, accuracy, and resolution (granularity) of scRNA-seq cell-type annotations across independent bioinformatics teams and pipelines.

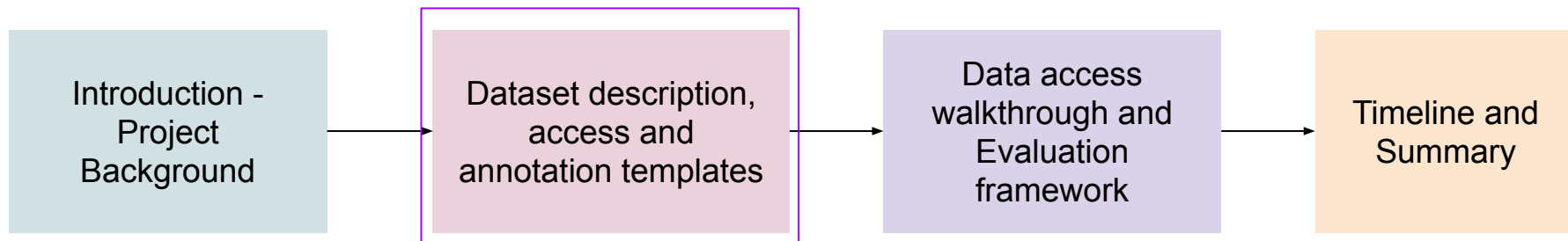
→ Build a multi-expert reference of cell-type annotations!



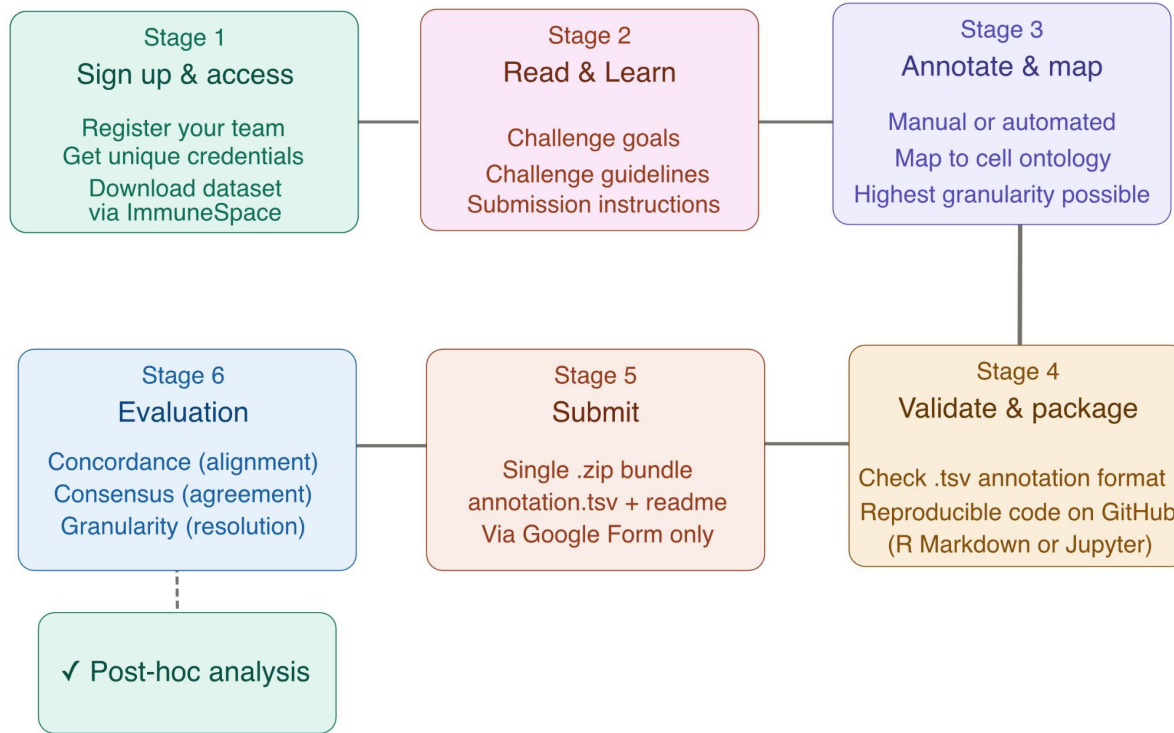
	A	B	C	D	E	F
1	<b>Celltype</b>	<b>cl: ID</b>	<b>Parent Class</b>	<b>isTerminalNode</b>	<b>Definitions</b>	
2	Blood Cell	CL:0000000	None	FALSE	A material entity of anaton	
3	Platelet	CL:0000233	Blood Cell	TRUE	A non-nucleated disk-shap	
4	RBC	CL:0000232	Blood Cell	TRUE	A red blood cell. In mamm	
5	HSC	CL:0000037	Blood Cell	TRUE	A stem cell from which all	
6	Doublet	None	Blood Cell	TRUE	An often technical artifact	
7	Leukocyte	CL:0000738	Blood Cell	FALSE	An achromatic cell of the r	
8	Lymphoid Cell	CL:0000542	Leukocyte	FALSE	A lymphocyte is a leukocy	
9	NK Cell	CL:0000623	Lymphoid Cell	FALSE	A lymphocyte that can spe	
10	T Cell	CL:0000084	Lymphoid Cell	FALSE	A type of lymphocyte who	
11	CD4 T Cell (ab)	CL:0000624	T Cell	FALSE	A mature alpha-beta T cel	
12	CD4 Naive / T Central Memory	CL:0000895	CD4 T Cell (ab)	TRUE	An antigen inexperienced	

- Tree contains broad lineages (ex. Lymphoid, Myeloid) and high-resolution subtypes (Conventional DC 1, plasmablast).
- Use the Nomenclature in the Cell-type Tree to ensure naming consistency
- Go for highest level of resolution you can support with confidence

## HIPC scRNA-seq Annotation Benchmark Challenge



# Overall Workflow



All resources are available at:

<https://immunespace.org/hipc-scna-seq-annotation-benchmark-challenge/>

## Data collection

Vaccination and infection scRNA-seq data were collected from the HIPC immune signature data resource.

## Data Processing

nf/core-scrnaseq pipeline for CellRanger  
ImmuneSpace/scintegrator for quality control

## Metadata

Basic metadata collected from the original paper

## Data Overview

16 studies  
390 Subjects  
2,718,103 cells  
19,624 average features

### Initial Data



### Filtering of Cells and Genes



Min. 400 genes  
Min. 3 cells

### QC Matrices



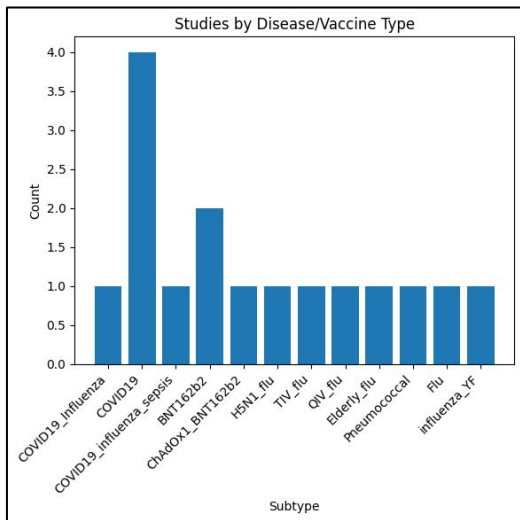
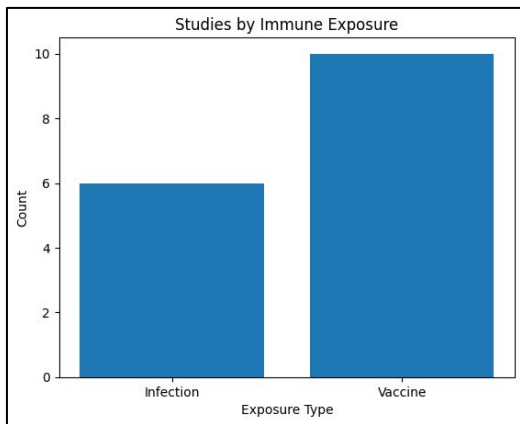
MT% < 15  
Total Counts > 200

### Filtered Data



subject id, sample id,  
status (timepoints),  
tissue, age, sex, n\_genes,  
total\_counts,  
ptc\_counts\_mt

# Dataset overview and update based on Participants' feedback



## Only processed data is provided

HIPC scRNA-seq Annotation Benchmark Challenge



yyasumizu

11d

Hi there,

I noticed that the provided data has been processed. Some h5ad/rds didn't include raw counts, and genes were filtered. In some data, only about 1200 genes were retained. I believe at least raw counts of all genes are needed for the analysis. Could you check the data?

Best,

Yoshi



Datasets with raw count data (13/16) - Ready	Studies where FASTq files where available	Infection_study_01, Infection_study_02, Infection_study_03, Infection_study_04, Infection_study_6, vaccination_study_01, vaccination_study_02, vaccination_study_03, vaccination_study_04, vaccination_study_05, vaccination_study_06, vaccination_study_07, vaccination_study_08
Datasets with raw count data (3/16) - Pending	The author provided raw count data	Infection_study_07, vaccination_study_09, vaccination_study10

## Dataset summary



### Index of /hipc-sc-comp-data/team01/

../	
<a href="#">fake_submission/</a>	26-Mar-2026 23:01
<a href="#">recipe_data/</a>	26-Mar-2026 23:09
<a href="#">CT_Ontology_Spreadsheet_20260323.xlsx</a>	26-Mar-2026 23:30
<a href="#">CT_tree_20260323.png</a>	26-Mar-2026 23:30
<a href="#">fake_submission.zip</a>	27-Feb-2026 23:46
<a href="#">readme.txt</a>	26-Mar-2026 23:42
<a href="#">study_annotation_template.tsv</a>	27-Feb-2026 21:46

<https://immunespace.org/hipc-sc-comp-data/>

submission\_bundle.zip

annotation.tsv    One file per dataset

cell\_barcode

AAACCTGAGAAACCTA-1

AAACCTGAGAAGGCCT-1

AAACCTGAGACAGACC-1

AAACCTGAGACGACGT-1

predicted\_cell\_type

CD4-positive T cell

classical monocyte

naive B cell

plasmacytoid DC

Labels must match CT\_Ontology\_Spreadsheet\_\*.xlsx exactly

readme.docx

Detailed methodology

Software versions

Manual intervention steps

GitHub repo link

R Markdown or Jupyter notebooks etc.

../

[Fake Pipeline ApproachSummary.docx](#)

[vaccination study 01 annotation fakePipeline.tsv](#)

[vaccination study 03 annotation fakePipeline.tsv](#)

[vaccination study 04 annotation fakePipeline.tsv](#)

[vaccination study 06 annotation fakePipeline.tsv](#)

[vaccination study 07 annotation fakePipeline.tsv](#)

27-Feb-2026 22:03

27-Feb-2026 21:54

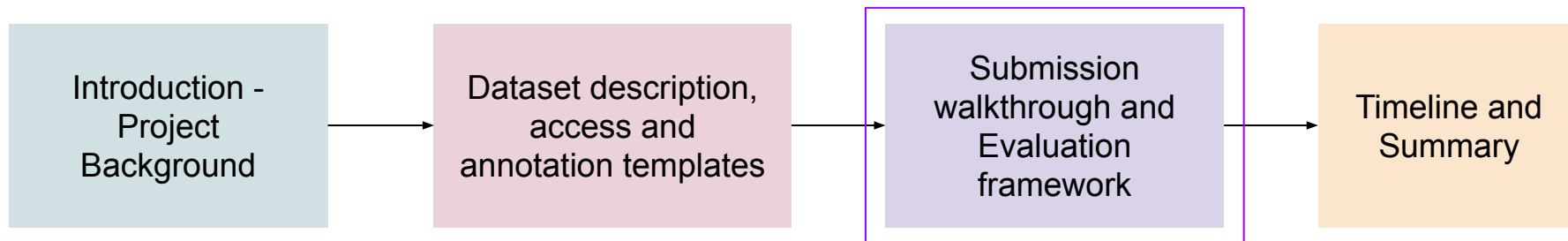
27-Feb-2026 21:56

25-Feb-2026 01:52

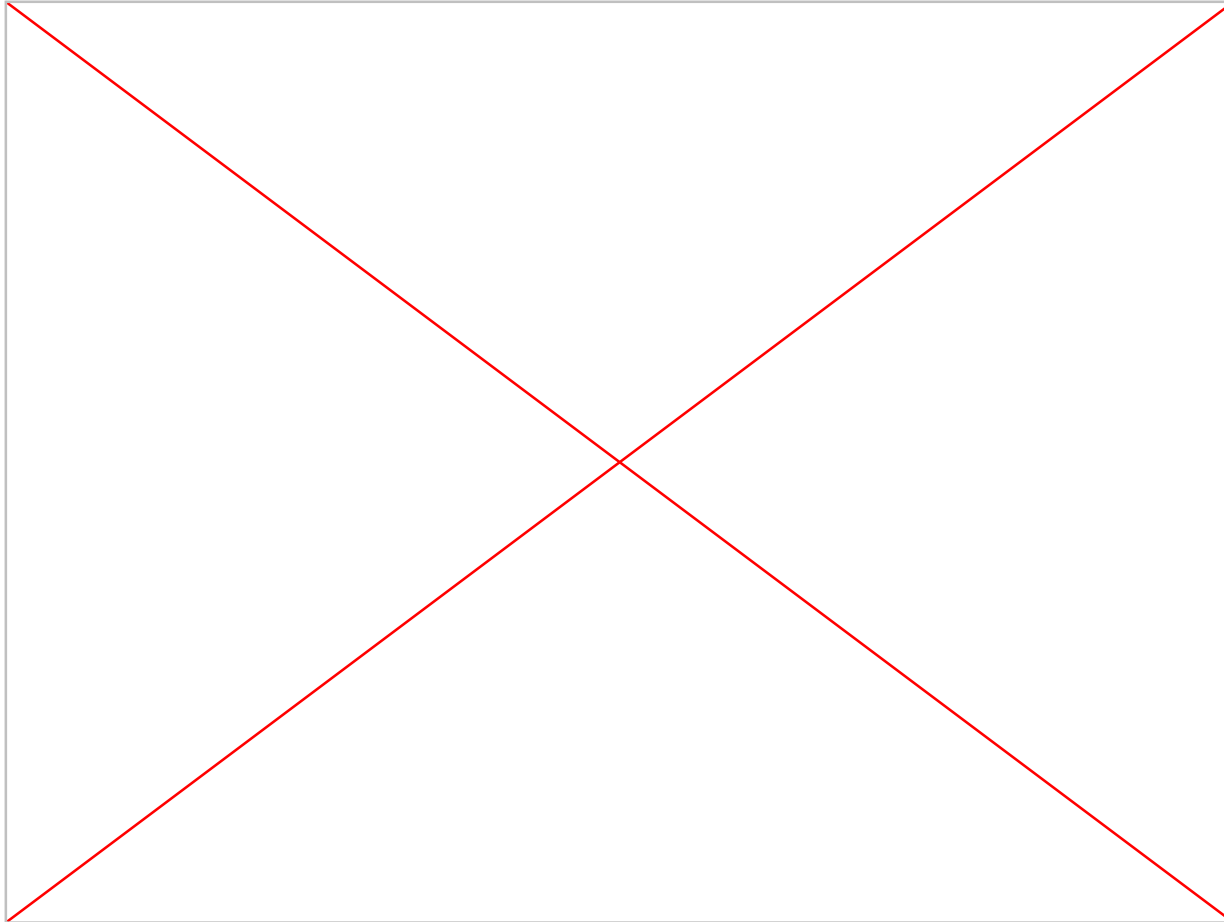
25-Feb-2026 01:52

25-Feb-2026 01:52

## HIPC scRNA-seq Annotation Benchmark Challenge



The screenshot shows a web browser window with a productivity dashboard. The browser's address bar displays the URL `https://immunespace.org/hipc-sc-comp-data/team02/`. The dashboard features a large background image of a mountain range at sunset. In the center, a large digital clock shows **12:33**, followed by the text **Good afternoon, Siddharth.** and a prompt: **What is your main goal for today?** Below this prompt is a horizontal line for input. In the top right corner, the weather is shown as **0m** and **23°** in San Diego, with the status **Focused Today**. A sidebar on the right contains an **Inbox** section with a **New Task** button and a **Tasks** section with a checked checkbox. At the bottom of the dashboard, there is a **Momentum** section and a **Customize Chrome** button. The Windows taskbar at the very bottom shows the search bar, taskbar icons, and the system tray with the time **12:33 PM** and date **4/5/2026**.



## scCRAFT+

### Index of /hipc-sc-comp-data/team01/recipe\_data/infection\_study\_01/

<a href="#">../</a>		
<a href="#">infection_study_01_QC_report.pdf</a>	04-Mar-2026 00:09	36423
<a href="#">infection_study_01_annotation_template.tsv</a>	04-Mar-2026 00:09	1130433
<a href="#">infection_study_01_processed.h5ad</a>	04-Mar-2026 00:09	916325484

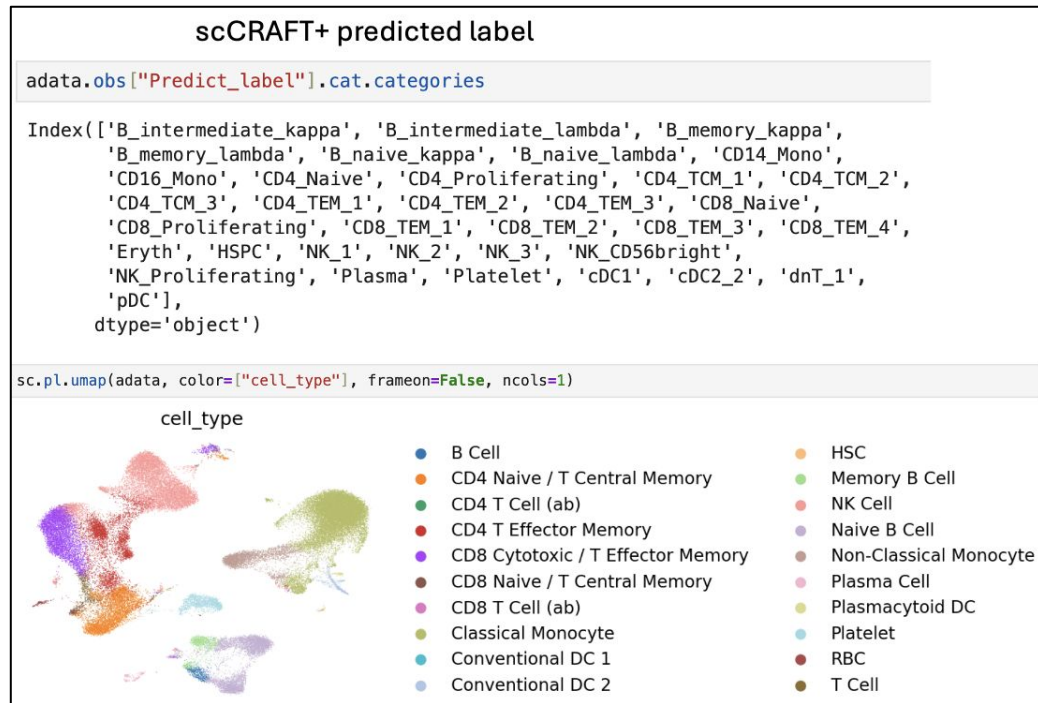
```
AnnData object with n_obs × n_vars = 54924 × 23226
  obs: 'sample_id', 'subject_id', 'arm', 'species', 'tissue', 'age', 'biological_sex', 'n_genes', 'n_genes_by_counts',
'total_counts', 'total_counts_mt', 'pct_counts_mt'
  var: 'gene_ids', 'feature_types', 'n_cells', 'mt', 'n_cells_by_counts', 'mean_counts', 'pct_dropout_by_counts', 'total_counts'
```

## scCRAFT+ auto-annotation

Annotate the dataset with Azimuth PBMC cell type list based on the marker list on their website

Map the Azimuth list to the ontology tree manually

1. Map the “Predict\_label” from *scCRAFT+* output to the given ontology tree “cell\_type”
2. Output the “cell\_type” with the corresponding barcode to a tsv file
3. Store all the tsv file for all datasets, compress the directory and submit



## Annotation consistency evaluation

### Goal:

Evaluate the consistency of cell type annotations across multiple sources.

### Agreement metric (Fleiss' $\kappa$ )

- Measures agreement among multiple annotators beyond chance
- $\kappa = 1$ : perfect agreement
- $\kappa = 0$ : agreement equals random chance
- Provides a global measure of annotation consistency

## Evaluator evaluation

Quantify how each annotator influences overall agreement and identifies potential inconsistencies.

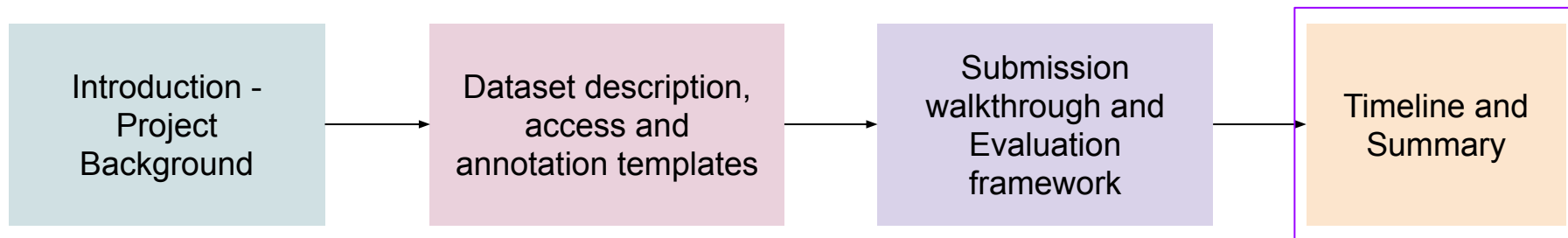
### Key idea (Leave-One-Out evaluation)

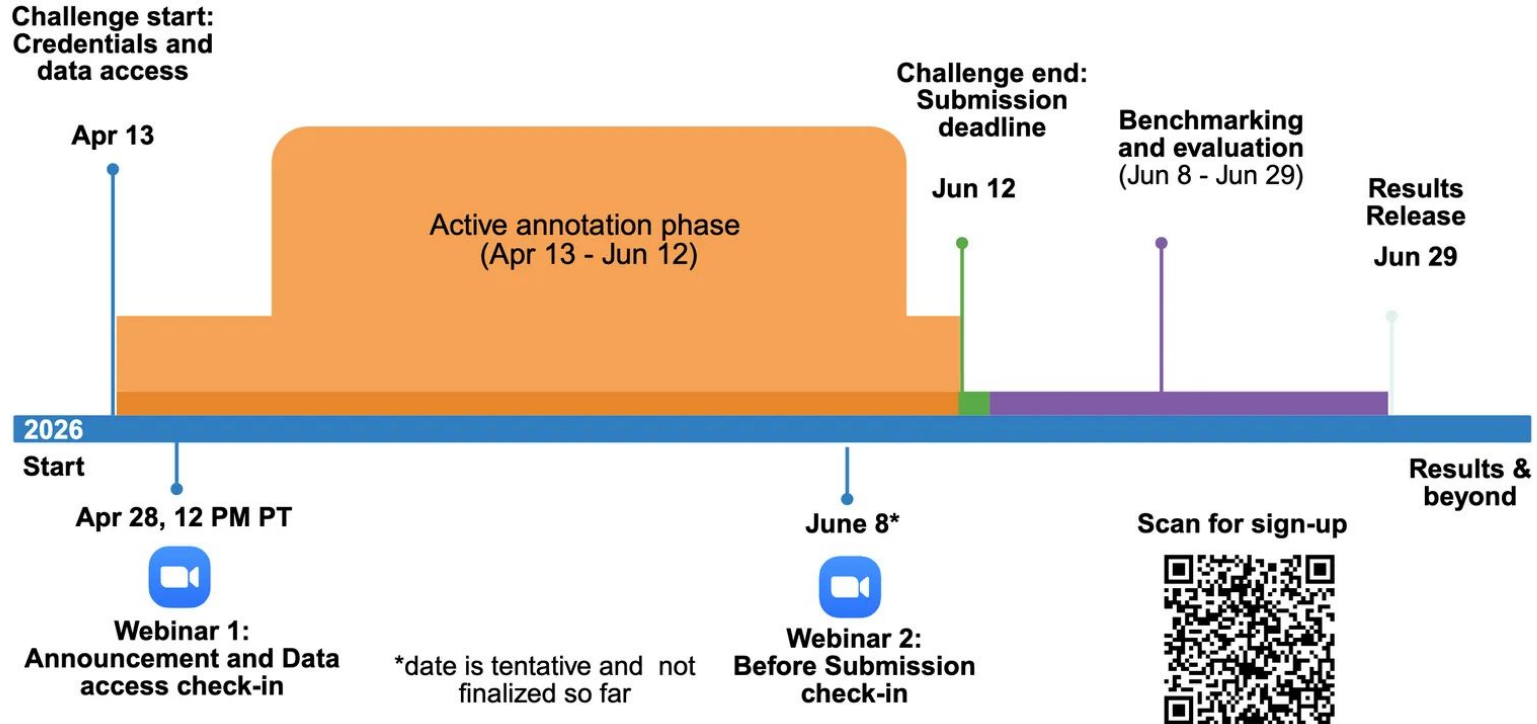
- Remove one annotator at a time
- Recompute agreement using the remaining annotators
- Compare to the full agreement
- If agreement increases after removal  $\rightarrow$  that annotator may reduce consistency
- If agreement decreases  $\rightarrow$  that annotator aligns well with others

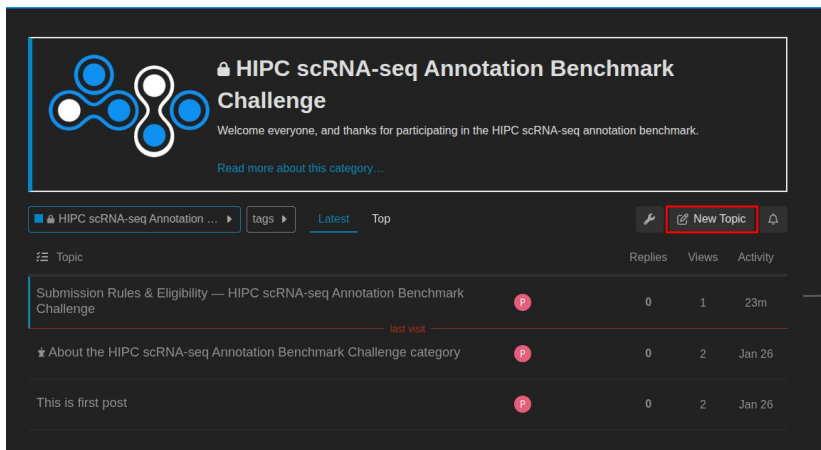
### Per-cell-type analysis

- Perform evaluation at each ontology node (cell type level)
- Capture fine-grained differences across the hierarchy

## HIPC scRNA-seq Annotation Benchmark Challenge







Topic	Replies	Views	Activity
Submission Rules & Eligibility — HIPC scRNA-seq Annotation Benchmark Challenge	0	1	23m
★ About the HIPC scRNA-seq Annotation Benchmark Challenge category	0	2	Jan 26
This is first post	0	2	Jan 26

We will be using Immunespace Solution Center for QA.  
 Use your Immunespace account to ask questions.

<https://discuss.immunespace.org/c/hipc-scRNA-seq-annotation-benchmark-challenge/42>

## Submission Rules & Eligibility — HIPC scRNA-seq Annotation Benchmark Challenge

HIPC scRNA-seq Annotation Benchmark Challenge

**pshinde** 24m

Entries are accepted throughout the challenge period (). Please read the following rules carefully before submitting.

### General Rules

Submissions are accepted from registered teams only. To participate, teams must sign up via the challenge registration form prior to submitting. Each team must have a designated team lead, who will receive login credentials and is responsible for the final submission.

A submission is defined as a single attempt encompassing annotations for all assigned datasets. Each team may submit only once. If a group wishes to submit multiple independent attempts (e.g., using different pipelines or methods), each attempt must be registered as a separate team.

Each team must annotate only their assigned studies and should not work on datasets assigned to other teams. To preserve the integrity of the benchmark, please do not discuss your assigned studies or annotations with other participating teams.


### Technical Requirements

All cell type labels must be mapped to the standardized Cell Ontology dictionary provided in `CT_ontology_Spreadsheet_*.xlsx`. Annotations that do not match the required nomenclature will not be evaluated. All entries must include fully documented, runnable code (R Markdown or Jupyter Notebook) hosted on GitHub. Submissions without reproducible workflows cannot be accepted.

### How to Submit

When ready, submit your final bundle via the secure Google Form. Submissions via email are not accepted.

For questions related to data access or cell ontology, please write your queries here in the comments.

 Reply

- The challenge starts April 13, 2026: Register to receive credentials and dataset access
  - Participants annotate immune cell types from scRNA-seq data using any manual or automated pipeline, mapping to the provided Cell Ontology tree at the highest possible granularity
  - Submissions (annotation.tsv + readme + GitHub repo) are due by June 13, 2026
  - Benchmarking and results release by June 29, 2026
- 
- All resources (datasets, ontology tree, submission templates, and guidelines) are available at: <https://immunespace.org/hipc-scRNA-seq-annotation-benchmark-challenge/>
  - Questions? Use the [ImmuneSpace Solutions Center](#).

# Thank You!

We are looking for submissions.

If you want to test your annotation pipelines against a rigorously curated ground truth of high-impact studies, this is the perfect opportunity to benchmark your methods/pipelines.

# Q&A Session



# HIPC scRNA-seq Annotation Benchmark Challenge

2nd Webinar: June 8 or 9, 2026

HIPC Coordinating Center

# Plans and TODOS

1. Data Set Update, March 27th finalized

2. Announcements (Pramod):

2.a Challenge Announcement: March 31 [depending on when the webinar poster is ready]

2.c Webinar: March 30/31 or April 7/8

2.b Challenge Start Date: April 7 or April 14

3. Pramod (Jian):

3.a Data Descriptions

3.b Data Access and download

3.c Submission Format

3.d Submission Example

4. Sid: Cell Ontology Tree, CSV and Explanations

5. Sid and Chuan: A small recording on data access and submission walkthrough

6. Chuan: 6.a Evaluation pipeline/structure and metric illustration; 6.b A given example with updated cell ontology tree