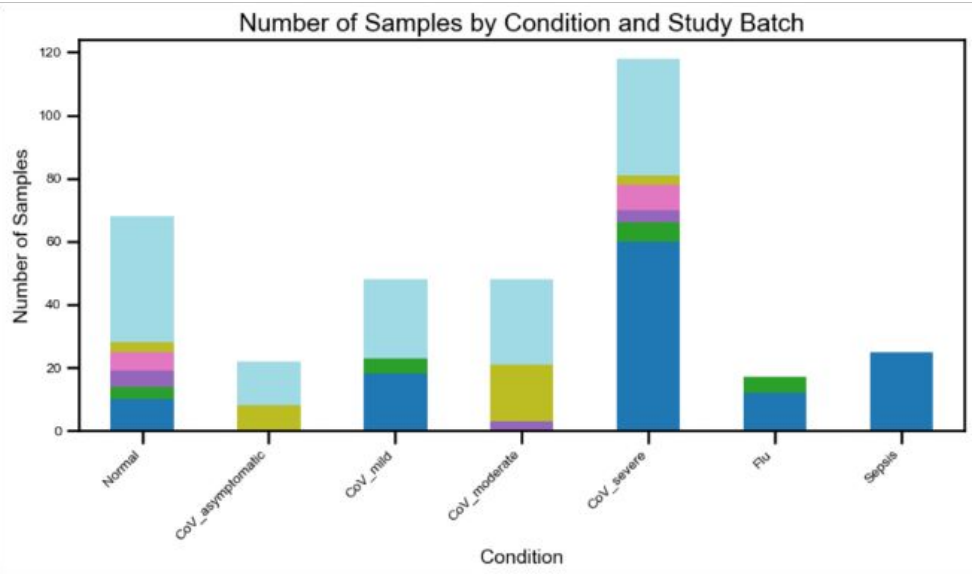
HIPC scRNA-seq Annotation Benchmark Challenge

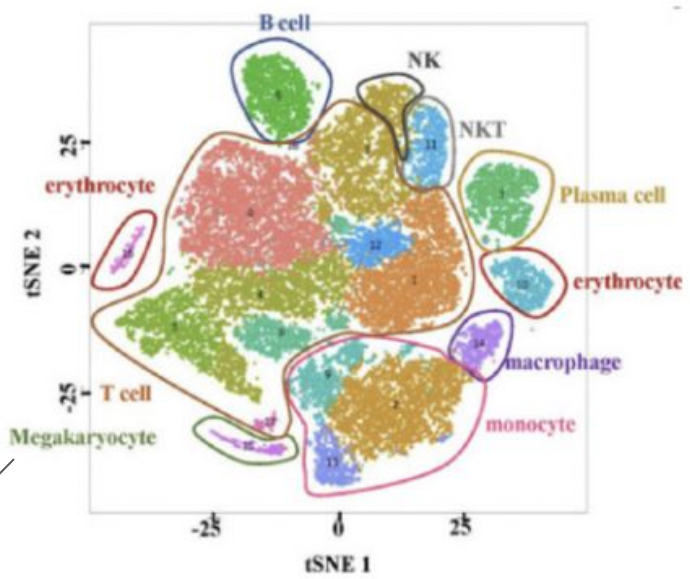
1st Webinar: April 28, 2026

HIPC Coordinating Center

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

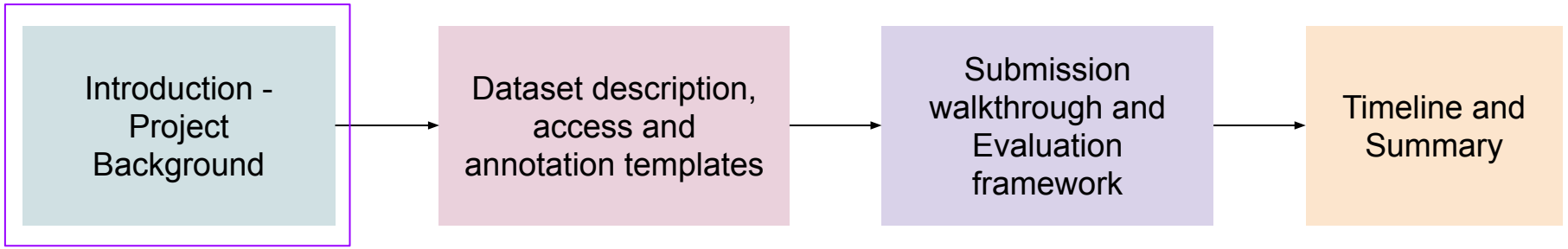
HIPC (Human Immunology Project Consortium): Pooling infection and vaccination resources for cell-resolution understanding under more broader contexts.





Reliability and reproducibility from such meta-analysis?

HIPC scRNA-seq Annotation Benchmark Challenge



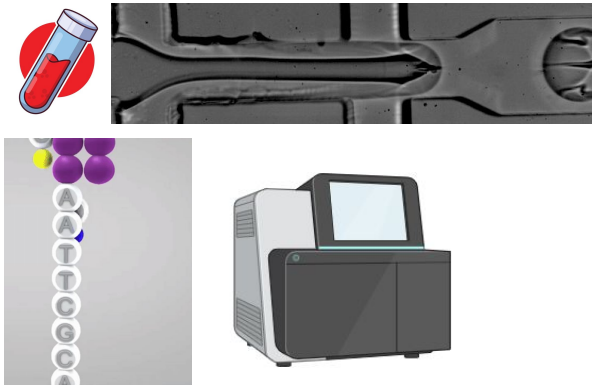
Siddharth Gaywala

Pramod Shinde,
Jian Xing

Chuan He,
Siddharth Gaywala

Pramod Shinde

Immune cell-type annotation: Background



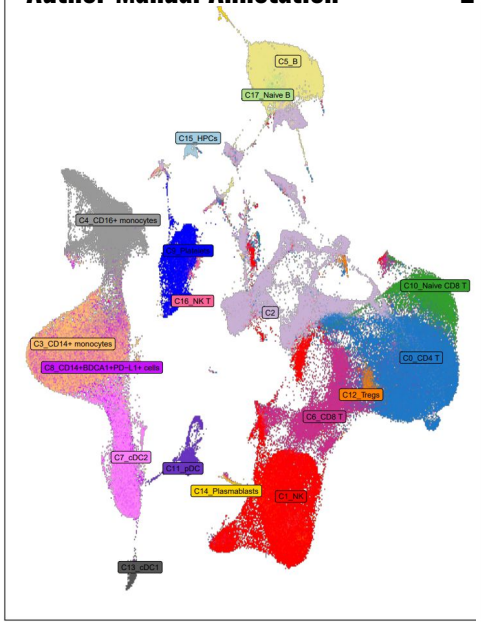
agg.TCR	7	.	7	9	33	8	.	12	29	.
agg.BCR	.	.	.	.	.	.	.	.	.	.
PF4	.	.	.	.	.	.	.	.	.	.
CD14	.	2	.	1	.	.	11	.	.	7
HBB	.	.	.	.	.	.	.	.	.	.
MT-CO2	44	56	16	51	58	21	21	38	46	89
SAMD11	.	.	.	.	.	.	.	.	.	.
NOC2L	.	.	.	.	1	.	1	.	.	.
KLHL17	.	.	.	.	.	.	.	.	.	.
PLEKHN1	.	.	.	.	.	.	.	.	.	.
PERM1	.	.	.	.	.	.	.	.	.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

- **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)

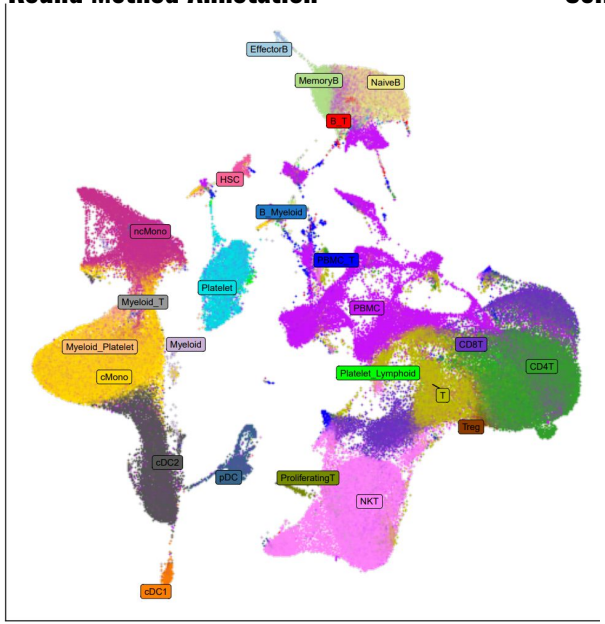
Immune cell-type annotation - Analysis & Workflow Challenges

- 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

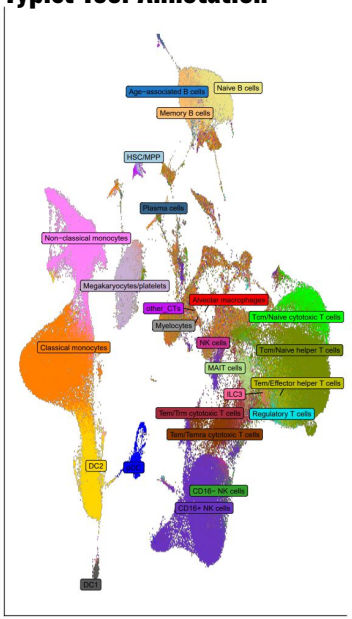
Author Manual Annotation



2-Round Method Annotation



Cell Typist Tool Annotation

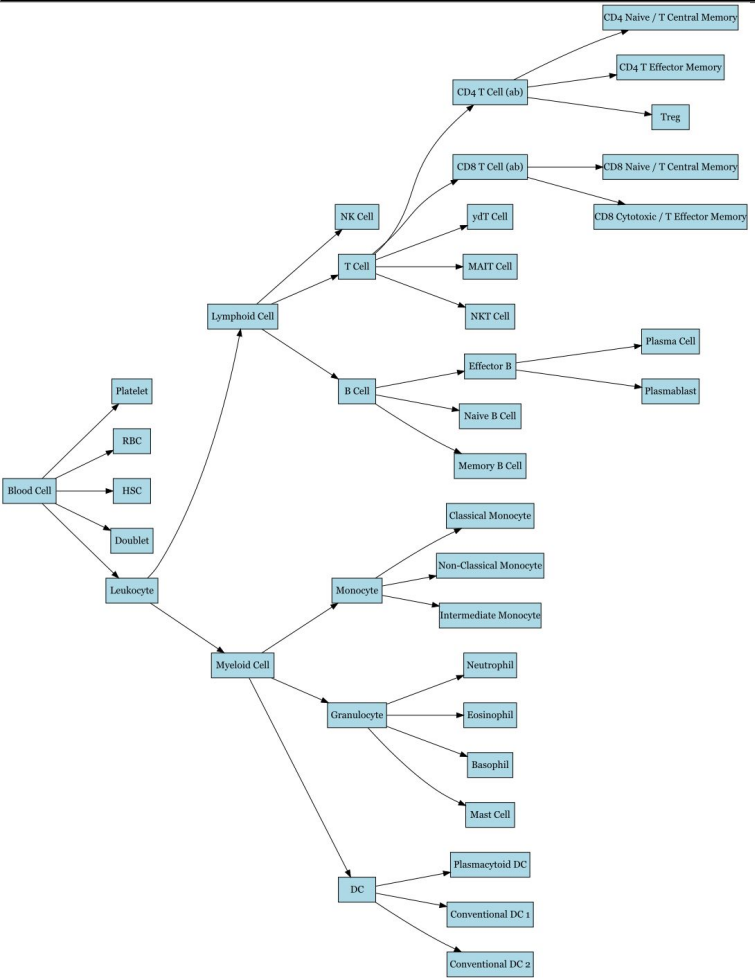


Goal: Evaluate the consistency, accuracy, and resolution (granularity) of scRNA-seq cell-type annotations across independent bioinformatics teams and pipelines.

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

Celltype Annotation Tree

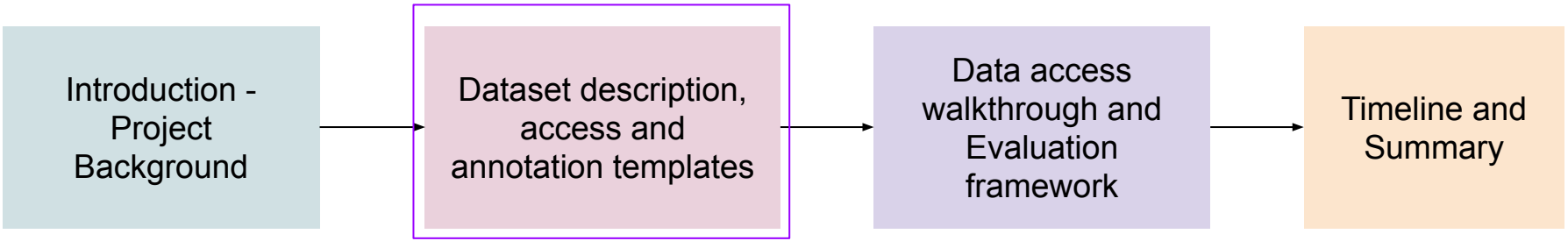
HIPC
scRNA-seq Annotation
Benchmark Challenge



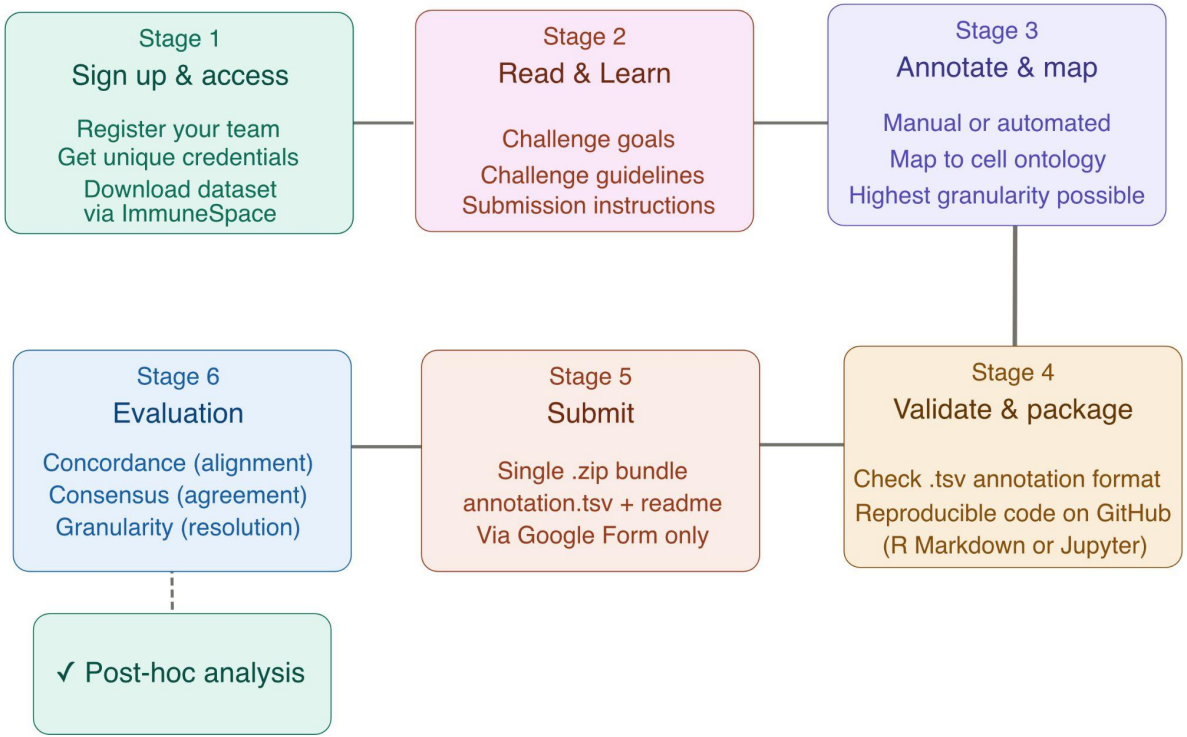
	A	B	C	D	E	F
1	**Celltype**	**cl: ID**	**Parent Class**	**isTerminalNode**	**Definitions**	
2	Blood Cell	CL:0000000	None	FALSE	A material entity of anaton	
3	Platelet	CL:0000233	Blood Cell	TRUE	A non-nucleated disk-shap	
4	RBC	CL:0000232	Blood Cell	TRUE	A red blood cell. In mamm	
5	HSC	CL:0000037	Blood 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 spo	
10	T Cell	CL:0000084	Lymphoid Cell	FALSE	A type of lymphocyte whos	
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-scrna-seq-annotation-benchmark-challenge/

HIPC
scRNA-seq Annotation Benchmark Challenge

Data collection

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

Data Processing

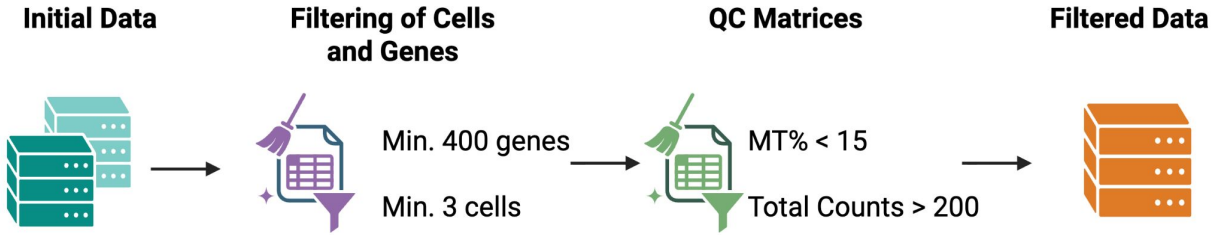
nf/core-scrnaseq pipeline for CellRanger
ImmuneSpace/scintegrator for quality control

Metadata

Basic metadata collected from the original paper

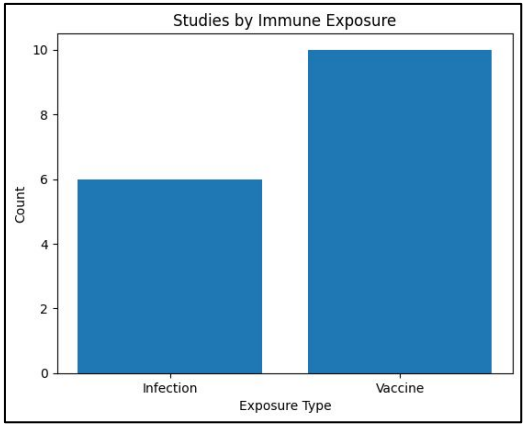
Data Overview

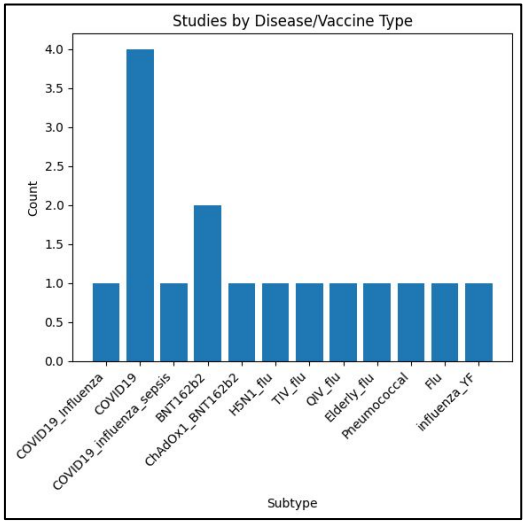
16 studies
390 Subjects
2,718,103 cells
19,624 average features

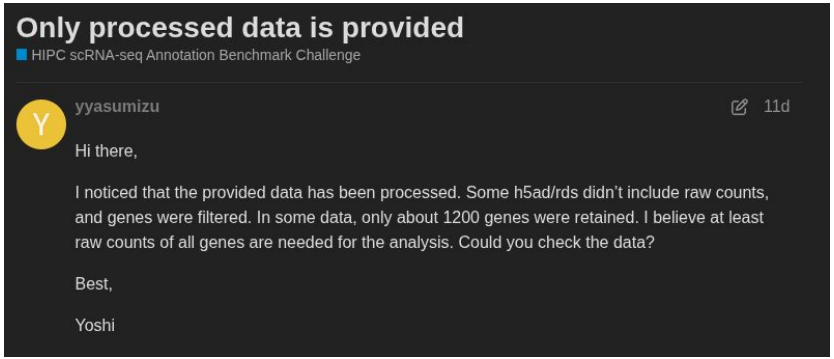


subject id, sample id, status (timepoints), tissue, age, sex, n_genes, total_counts, ptc_counts_mt

Dataset overview and update based on Participants' feedback







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

Website and data access

IMMUNESPACE

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

../		
fake_submission/	26-Mar-2026	23:01
recipe_data/	26-Mar-2026	23:09
CT_Ontology_Spreadsheet_20260323.xlsx	26-Mar-2026	23:30
CT_tree_20260323.png	26-Mar-2026	23:30
fake_submission.zip	27-Feb-2026	23:46
readme.txt	26-Mar-2026	23:42
study_annotation_template.tsv	27-Feb-2026	21:46

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

Data submission format

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submission_bundle.zip

annotation.tsv — One file per dataset

cell_barcode	predicted_cell_type
AAACCTGAGAAACCTA-1	CD4-positive T cell
AAACCTGAGAAGGCCT-1	classical monocyte
AAACCTGAGACAGACC-1	naive B cell
AAACCTGAGACGACGT-1	plasmacytoid DC

Labels must match CT_Ontology_Spreadsheet_*.xlsx exactly

readme.docx

Detailed methodology

Software versions

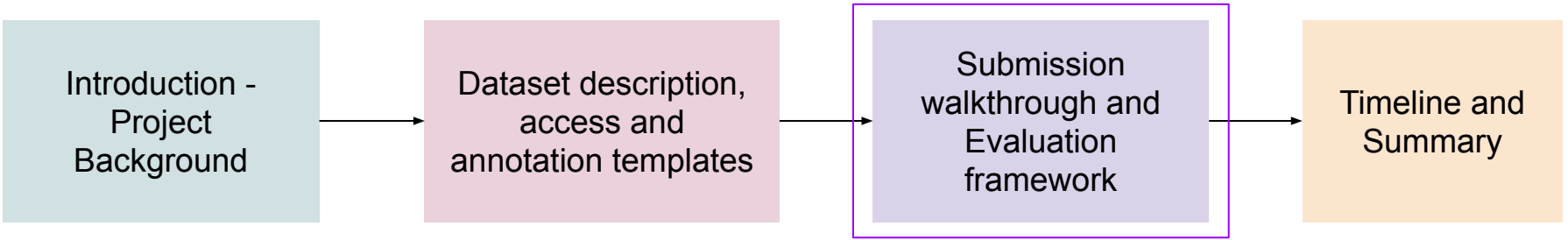
Manual intervention steps

GitHub repo link

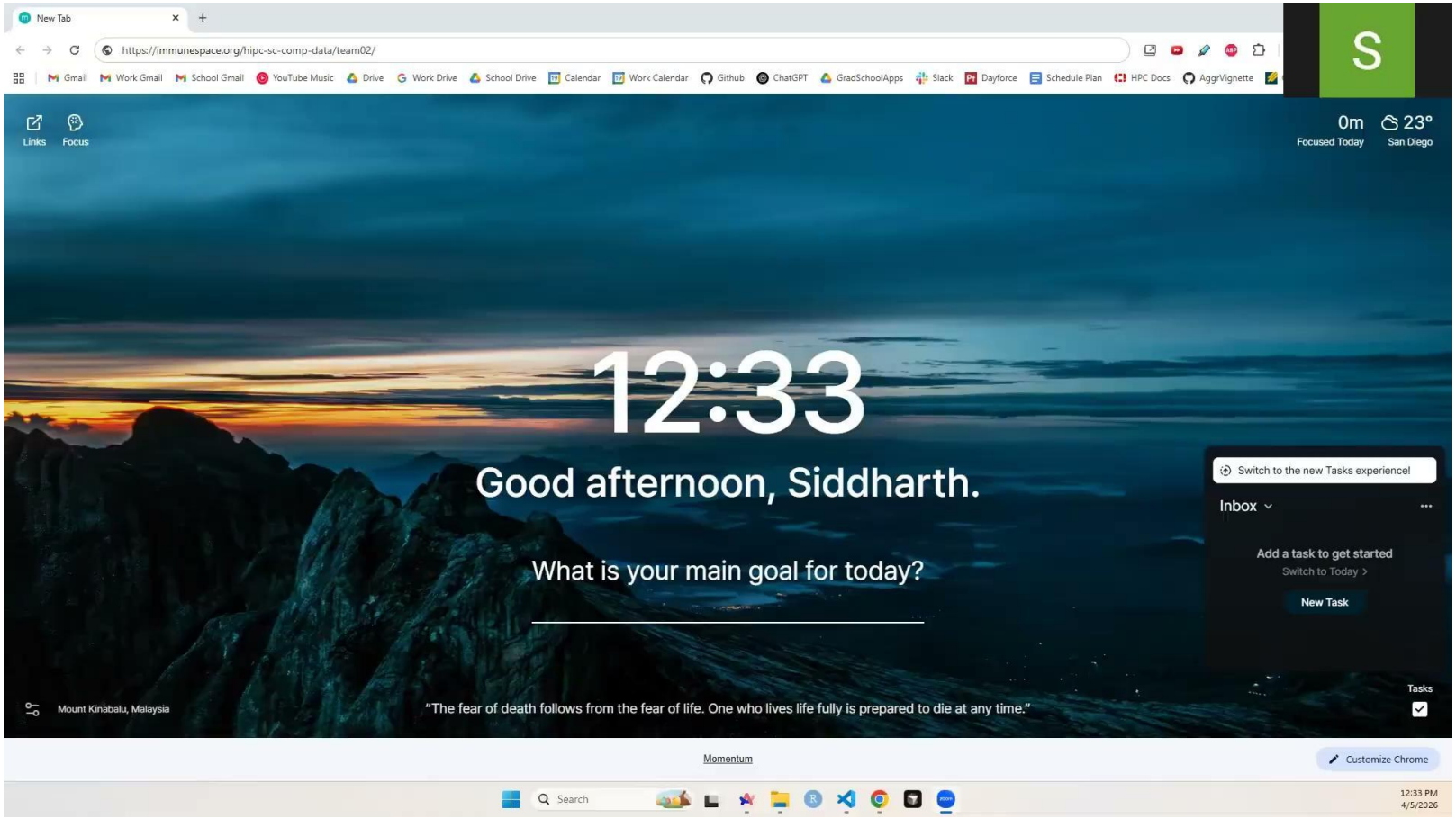
R Markdown or Jupyter notebooks etc.

```
../
Fake_Pipeline_ApproachSummary.docx                  27-Feb-2026 22:03
vaccination_study_01_annotation_fakePipeline.tsv    27-Feb-2026 21:54
vaccination_study_03_annotation_fakePipeline.tsv    27-Feb-2026 21:56
vaccination_study_04_annotation_fakePipeline.tsv    25-Feb-2026 01:52
vaccination_study_06_annotation_fakePipeline.tsv    25-Feb-2026 01:52
vaccination_study_07_annotation_fakePipeline.tsv    25-Feb-2026 01:52
```

HIPC scRNA-seq Annotation Benchmark Challenge



Data access and submission walkthrough



Data access and submission walkthrough

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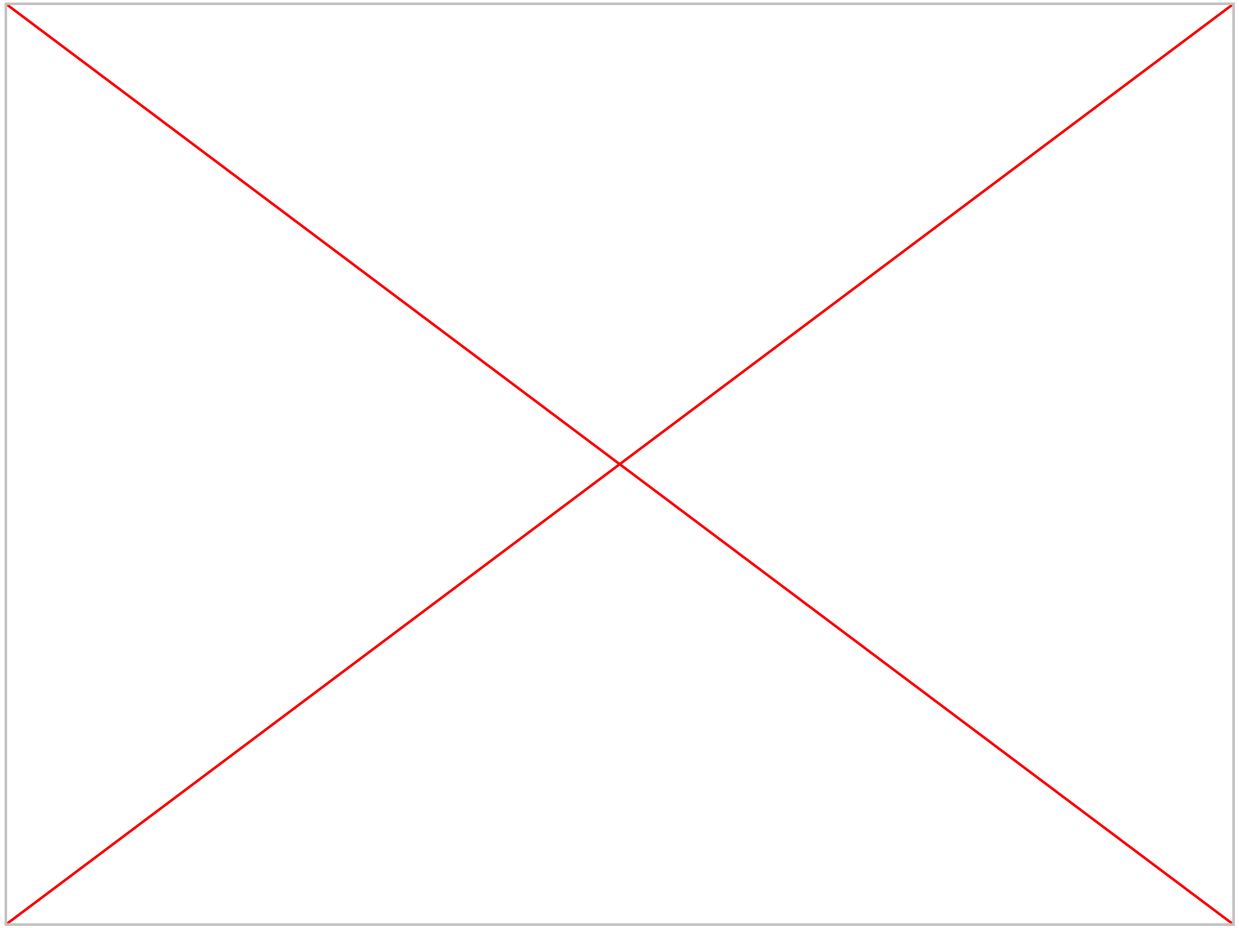


scCRAFT+

Index of /hipc-sc-comp-data/team01/recipe_data/infection_study_01/

../		
infection_study_01_QC_report.pdf	04-Mar-2026 00:09	36423
infection_study_01_annotation_template.tsv	04-Mar-2026 00:09	1130433
infection_study_01_processed.h5ad	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

Example: scCRAFT+ based annotation approach

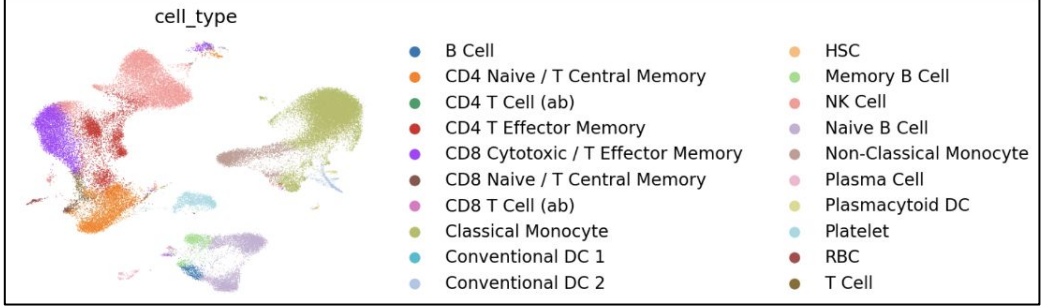
1. Map the “Predict_label” from *scCRAFT+* output to the given ontology tree “cell_type”
2. Output the “cell_type” with the corresponding barcode to a tsv file
3. Store all the *tsv* file for all datasets, compress the directory and submit

scCRAFT+ predicted label

```
adata.obs["Predict_label"].cat.categories
```

```
Index(['B_intermediate_kappa', 'B_intermediate_lambda', 'B_memory_kappa',
       'B_memory_lambda', 'B_naive_kappa', 'B_naive_lambda', 'CD14_Mono',
       'CD16_Mono', 'CD4_Naive', 'CD4_Proliferating', 'CD4_TCM_1', 'CD4_TCM_2',
       'CD4_TCM_3', 'CD4_TEM_1', 'CD4_TEM_2', 'CD4_TEM_3', 'CD8_Naive',
       'CD8_Proliferating', 'CD8_TEM_1', 'CD8_TEM_2', 'CD8_TEM_3', 'CD8_TEM_4',
       'Eryth', 'HSPC', 'NK_1', 'NK_2', 'NK_3', 'NK_CD56bright',
       'NK_Proliferating', 'Plasma', 'Platelet', 'cDC1', 'cDC2_2', 'dnT_1',
       'pDC'],
      dtype='object')
```

```
sc.pl.umap(adata, color=["cell_type"], frameon=False, ncols=1)
```



Annotation consistency evaluation

Goal:
Evaluate the consistency of cell type annotations across multiple sources.

Agreement metric (Fleiss' κ)

- Measures agreement among multiple annotators beyond chance
- κ = 1: perfect agreement
- κ = 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 → that annotator may reduce consistency
- If agreement decreases → 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

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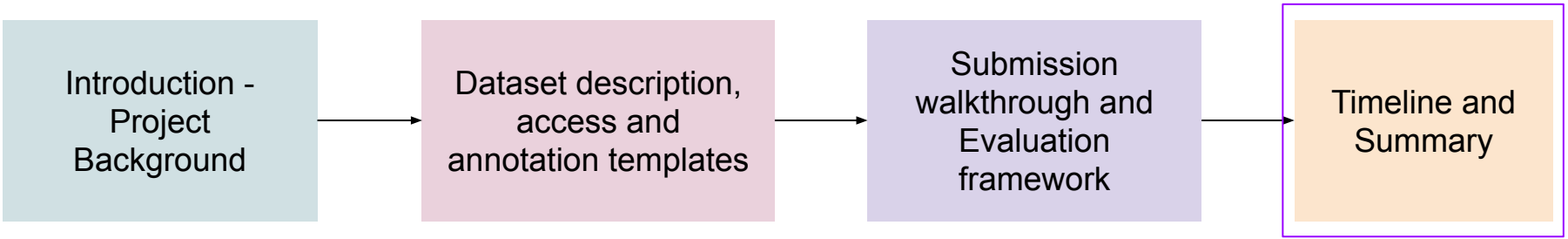
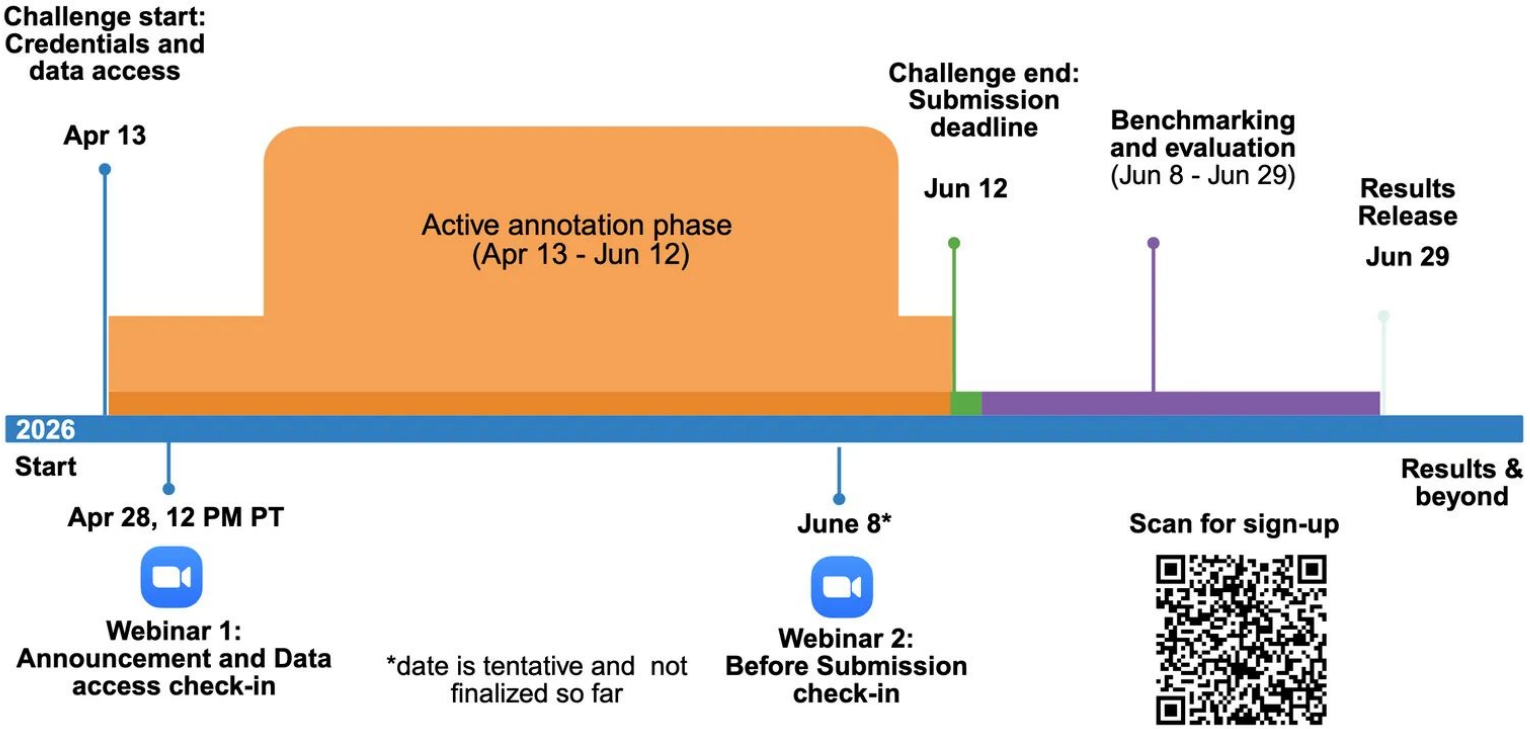


Timeline

HIPC
scRNA-seq Annotation
Benchmark Challenge



Solutions center for Q&As

HIPC
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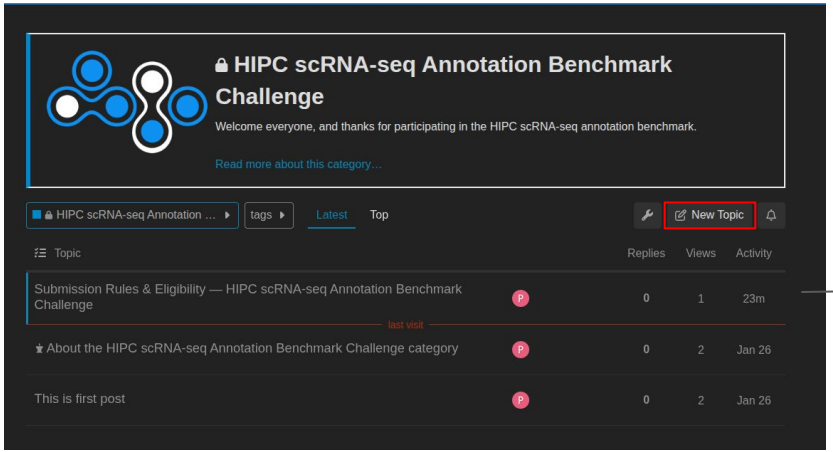
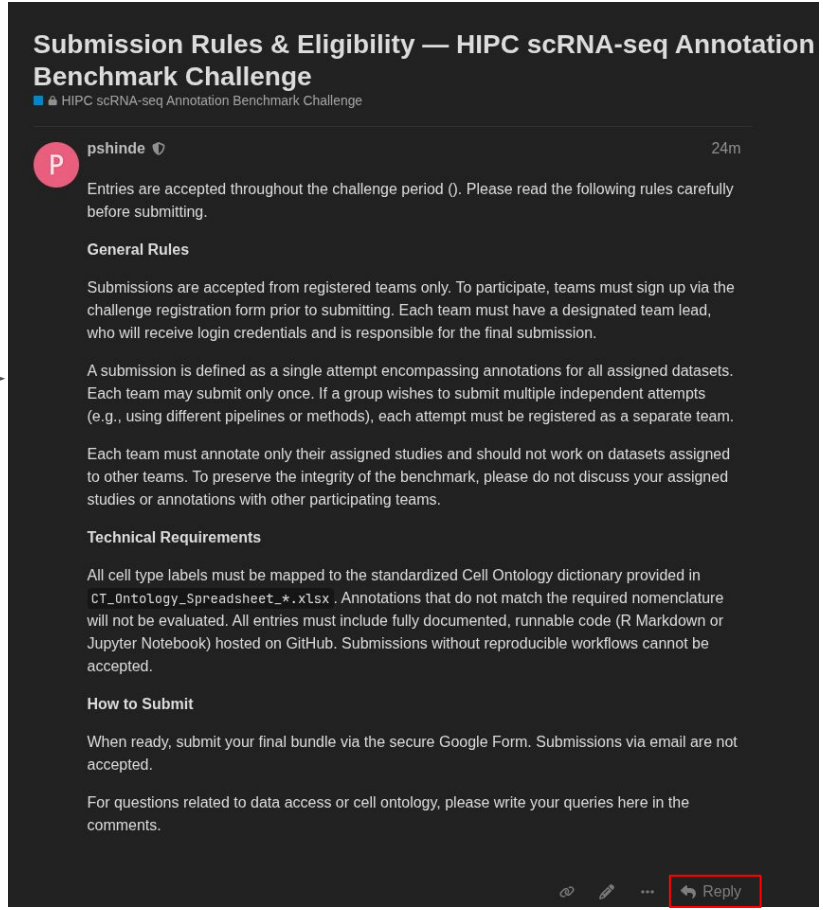
Benchmark Challenge





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

Key takeaways from this webinar

- 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