

MILRD Virtual Training Projects

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VTP OVERVIEW

Single-cell & Spatial Transcriptomics + Neuroinflammatory Astrocyte Characterization

Source Data

Hasel, P., Rose, I.V.L., Sadick, J.S. *et al.* Neuroinflammatory astrocyte subtypes in the mouse brain. [Nat Neurosci 24, 1475–1487 \(2021\)](#).
<https://doi.org/10.1038/s41593-021-00905-6>.

Aim

Transcriptomically profile Neuroinflammatory astrocyte subtypes in the mouse brain using single-cell RNA-sequencing (scRNA-seq), spatial transcriptomics and integrated analysis of previously published datasets

Tentative Schedule

Day 1 Schedule

9-10 am: Getting Started

- Introduction
- Platform login & orientation
- Project dataset background/orientation

10-11 am: scRNAseq Part 1 (Individual Samples)

- Alignment/Count generation
- Gene Count / UMI Review
- Doublet detection
- Mitochondrial Gene Contribution
- Filtration

11am-12pm: Discussion

12-1pm: Lunch Break

1-2pm: scRNAseq Part 2 (Individual Samples)

- Log normalization, variable feature identification & anchoring
- Dimensional reduction with PCA & t-SNE
- Marker detection
- Dotplot
- Initial Cell Type/Subtype Assignment

2-3pm: Discussion

3-4:30pm: Comprehensive Analysis (All Samples)

- Analysis with all samples from raw counts through anchored TSNE plots across all conditions

4:30-5pm ET: Closing Discussion

Day 2 Schedule

9-9:30 am: Getting Started

- Day 1 review

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9:30-10:30 am: Spatial Transcriptomics Analysis

- Load and normalize data
- Integrate samples based on variable features
- Identify/visualize cell clusters
- Highlight genes differentially expressed in identified scRNA-seq clusters

10:30am-12 pm: Discussion Session

12-1pm: Lunch Break

1-3:30 pm: Integration of External Datasets and scRNAseq

- QC, normalize, and integrate count matrices from outside studies
- Isolate astrocyte populations from each dataset, merge and reanalyze
- Compare results comparing the paper's acute inflammation model with models from the outside datasets (Alzheimer's disease model, an MS model, and stab wound injury model)

3:30-5 pm: Wrap-up Discussion

Learning Goals

<i>Discussion Topics</i>	<i>Bioinformatics Tasks + Methods</i>
<ul style="list-style-type: none">● Principles and Methods of scRNA-seq, spatial transcriptomics● scRNAseq Platform Comparison: inDrop, Drop-Seq, 10X Genomics● 2D projection mapping (e.g. <i>t</i>-SNE vs UMAP)● Principles and methods of scRNA-seq alignment, count generation, and file outputs● Multiple Dataset Integration and Label Transfer	<ul style="list-style-type: none">● Downloading data from public repositories● Reference indexing, alignment, counting in Linux terminal● Analysis with Seurat in R● Seurat Object Structure● Library QC● Normalization● Data filtering/clustering/PCA/<i>t</i>-SNE● Cell-type Assignment● Gene Expression● Merged Analysis and Differential Gene Expression Analysis● Integrated analysis

MILRD Provides

- ❖ Unlimited support from expert mentors
- ❖ Access to all required high-performance cloud-compute resources (AWS), analysis tools and software
- ❖ Access to all source data required to complete your project
- ❖ Optional Pre-VTP Preparation

Participants Provide

- ❖ A computer running Windows or MacOS
- ❖ Google Chrome, Safari, Firefox, or Edge
- ❖ A stable Internet connection