# Welcome to the December 2024 Advanced Single-cell RNA-seq Training Workshop!

WIFI: Bala Plaza Password: Bala123! December 10 - December 12, 2024 Childhood Cancer Data Lab <u>https://alexslemonade.github.io/2024-december-training/</u>

Childhood Cancer



# Tell us about you!

- What's your name?
- What are you studying?
- What is a recent piece of media (book, tv, movie, live performance...) you enjoyed?

### Meet your instructors



JOSH Joshua Shapiro

#### Data Scientist @ the Data Lab

PhD Ecology & Evolution, UChicago Postdoc Integrative Genomics, Princeton

Research interests:

- Evolutionary genomics
- Single cell workflows
- ) jashapiro

### Meet your instructors



STEPHANIE Stephanie Spielman

#### Data Scientist @ the Data Lab

PhD Integrative Biology UT Austin Postdoc Computational Molecular Evolution Temple

Research interests:

- Protein evolution and comparative sequence analysis
- Data science and bioinformatics education
- **3** sjspielman

### Meet your instructors



JACLYN Jaclyn Taroni

#### Director @ the Data Lab

PhD Genetics *Dartmouth* Postdoc Computational Biology *UPenn* 

Research interests:

- Transcriptomics in rare, complex diseases
- Unsupervised pattern extraction

jaclyn-taroni

## And of course!



Scientific Community Manager

- Helps administer Data Lab offerings such as workshops
- Manages communications
- Saves the rest of us from ourselves

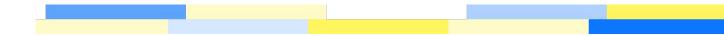
JEN Jen O'Malley

# Tell us about you!

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# Code of Conduct



# Be kind, have fun

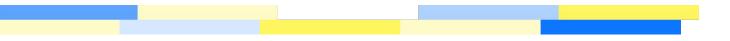
We value the involvement of everyone in the community. We are committed to creating a friendly and respectful place for learning, teaching, and contributing.

- Use welcoming and inclusive language
- Be respectful of different viewpoints and experiences
- Gracefully accept constructive criticism
- Focus on what is best for the community
- Show courtesy and respect towards other community members

Read the full Code of Conduct here:

https://alexslemonade.github.io/2024-december-training/code-of-conduct.html

If you at any time feel harassed or treated inappropriately, please contact <u>ccdl@alexslemonade.org.</u>



# What you will learn (and what you won't)



# What you will learn

- We will review working with single-cell RNA-seq data in R with Bioconductor.
- We will introduce working with CITE-seq data.
- We will address integration of multiple single-cell RNA-seq libraries.
- We will describe approaches to differential expression analyses with single-cell data.
- We will cover pathway analysis using GSEA and AUCell.

# What you won't learn

- We won't address experimental design (e.g., how many replicates you need).
- We won't compare tools (e.g., Seurat vs. Scater for single-cell RNA-seq data pre-processing).
- We won't cover pre-processing (alignment and quantification) of single-cell data.
- We won't address integrating with other omics types (e.g. joint analysis of single-cell RNA-seq and ATAC-seq).
- We won't cover every feature or assumption of the tools we do present, but we will try to highlight the features and gotchas that we think are relevant to most users.
- You may not be able to perform every analysis you need for your own work, particularly for complex experimental designs.

### How do we pick what we teach?

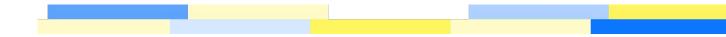
We want methods to be or to have:

- Useful for a wide range of experimental designs, sample sizes
- Easy to use, well-documented, and consistently updated
- Solid tutorials, a sizeable user base, and responsive authors/maintainers

We have a preference for methods that integrate easily into a single workflow that can be run on a laptop (and our own personal biases as scientists).



# Schedule



#### Tuesday

#### Workshop Intro

<u>Modules</u> -Reading, filtering, and normalizing scRNA-seq data -Integrating scRNA-seq datasets Wednesday

Module Differential expression analysis for scRNA-seq

<u>Consultations</u> -Exercise notebooks -Your own data Thursday

Module Pathway and gene-set analysis

<u>Consultations</u> -Exercise notebooks -Your own data

**Presentations** 

<u>Consultations</u> -Exercise notebooks

Full schedule: https://alexslemonade.github.io/2024-december-training/workshop/SCHEDULE.html

## **Daily Schedule Outline**

Instruction Full group Lectures

#### **Consultation Period**

Exercise notebooks Your own data

- Introduce concepts and background
- Demonstrate usage
- Answer general questions

- Ask questions of instructors and other participants
- Practice what you have learned
- Work on exercises individually or in groups
- Work with your own data

### Module Layout

- 01-read\_filter\_normalize\_scRNA-live.Rmd
- 02-dataset\_integration-live.Rmd
- 03-differential\_expression-live.Rmd
- 04-gene\_set\_enrichment\_analysis-live.Rmd
- 05-aucell-live.Rmd
- analysis 📄
- 📄 data
- diagrams
- exercise\_01-citeseq.Rmd
- exercise\_02-integration.Rmd
- exercise\_03-diffexp.Rmd
- exercise\_04-scrna\_pathway.Rmd
- exercise\_05-cluster\_evaluation.Rmd

These are Instruction notebooks.We'll walk through these together, adding code during the workshop.

These are Exercise notebooks.
Use these to practice what you have learned. We're here to help!

### Module cheatsheets cover key functions

#### dplyr

Read the dplyr package documentation here. A vignette on the usage of the dplyr package can be found here.

Library/Package	Piece of code	What it's called	What it does
dplyr	<u>\$&gt;\$</u>	Pipe operator	Funnels a data.frame through tidyverse operations
dplyr	filter()	Filter	Returns a subset of rows matching the conditions of the specified logical argument
dplyr	arrange()	Arrange	Reorders rows in ascending order. arrange(desc()) would reorder rows in descending order.
dplyr	<u>select()</u>	Select	Selects columns that match the specified argument
dplyr	mutate()	Mutate	Adds a new column that is a function of existing columns
dplyr	<u>summarise()</u>	Summarise	Summarises multiple values in an object into a single value. This function can be used with other functions to retrieve a single output value for the grouped values. summarize and summarise are synonyms in this package.
dplyr	rename()	Rename	Renames designated columns while keeping all variables of the data.frame
dplyr	<u>group_by()</u>	Group By	Groups data into rows that contain the same specified value(s)
dplyr	inner_join()	Inner Join	Joins data from two data frames, retaining only the rows that are in both datasets.

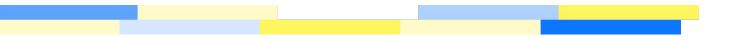
## **Thursday Afternoon**

Your own projects Exercise notebooks

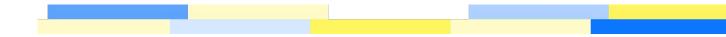
Spend Thursday afternoon working with your own data, getting assistance as needed from Data Lab staff and each other.

**Presentations** 

Present what you worked on during the consultation times to the group!



# **Training Procedures**



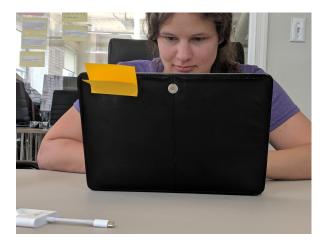
### We're going to use sticky notes and note cards...

- As an alternative to raising your hands for help
- To give feedback about the session

### During the session

Use the yellow sticky note to let us know that you need help or having some trouble following along.

One of our helpers will come over and help you.



Use the blue sticky note to let us know when a long-running step has completed.



### At the end of each session

At the end of each module, write down your muddiest point on a white note card: At the end of the session, use one blue note card and one white note card to write down:

I do not understand

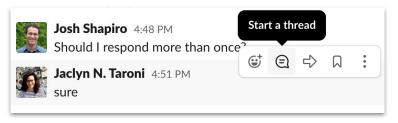
What did you like about this session?

How might we improve the session?



## We encourage you to use Slack

- You have been added to the **#2024-december-training** Slack channel
- Post public questions, get help with errors and debugging, make comments, and help others!
  - Use threads to keep related content together
- Stay in touch after the workshop!



## Housekeeping Notes

- Waivers If you have not yet done so, we have hard copy waivers available
- Where are the restrooms?
- Where is water/coffee available?
- Snacks!
- Dinner tomorrow (Wednesday) at 6:00PM at Couch Tomato located at 102 Rector St., Philadelphia, PA 19127