Welcome to the March 2023 Advanced Single-cell RNA-seq Training Workshop!

March 13 - March 17, 2023
Childhood Cancer Data Lab
https://alexslemonade.github.io/2023-march-training/

Childhood Cancer Data Lab x Alex's Lemonade Stand

Tell us about you!

- What's your name?
- What are you studying?
- Do you have any pets? If not, what kind of pet would you choose to have?

Meet your instructors

JOSH

Joshua Shapiro

Senior 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**

sjspielman

Meet your instructors

CHANTE

Chante Bethell

Biological Data Analyst @ the Data Lab

Bachelor’s in Bioinformatics from *Rowan University*

Research interests:

- **Functional motifs in the proteome**

cbethell

Meet your instructors

ALLY

Ally Hawkins

Data Scientist @ the Data Lab

PhD Cancer Biology *University of Michigan*
Postdoc Computational Biology *Cornell*

Research interests:

- **Single cell data analysis**
- **Origins of pediatric solid tumors**

allyhawkins

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

Other staff you may see (or have seen already!)

JEN

Jen O'Malley

Scientific Community Manager

- Helps administer Data Lab offerings such as training workshops
- Manages communications

DEEPA

Deepa Prasad

User Experience Designer

- Talks to researchers about their needs and frustrations
- Designs usable software

DAVID

David Mejia

Engineering Manager

- Makes websites
- Debugs

Tell us about you!

- What’s your name?
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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/2023-march-training/code-of-conduct.html

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

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 cover cell-type identification using SingleR and 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.

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

Monday

Workshop Intro

Module
Single-cell Analysis with R and Bioconductor

Consultations
-Exercise notebooks

Tuesday

Module
Identifying Cell Types with scRNA-seq Data

Consultations
-Exercise notebooks

Wednesday

Module
Integrating scRNA-seq datasets

Consultations
-Exercise notebooks
-Your own data

Thursday

Module
Differential expression analysis for scRNA-seq

Consultations
-Exercise notebooks
-Your own data

Friday

Consultations
-Exercise notebooks
-Your own data

Presentations

Full schedule: https://alexslemonade.github.io/2023-march-training/workshop/SCHEDULE.html

Daily Schedule Outline

Instruction
Full group
Lectures

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

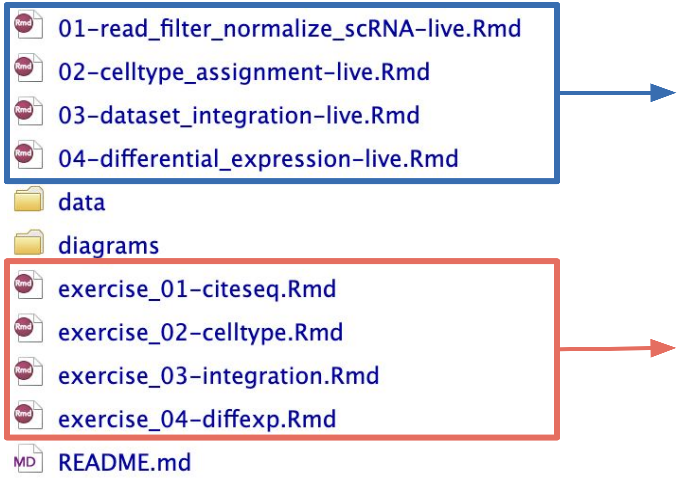
Breakout
Small groups
Start exercise notebooks

- Split up into Zoom breakout rooms
- Ask questions of instructors and other participants

Consultation Period
Exercise notebooks
Your own data

- Practice what you have learned
- Work on exercises individually or in groups
- Work with your own data

Module Layout

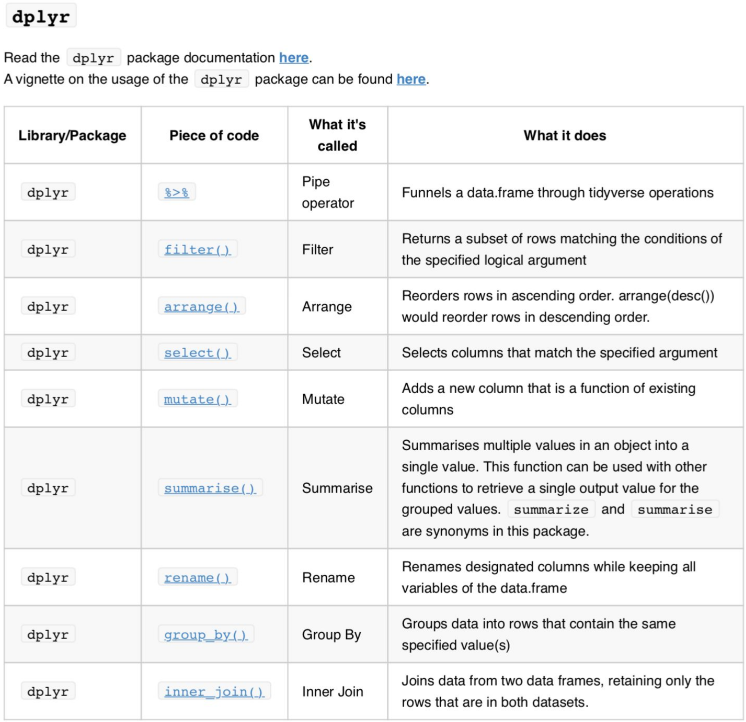


01-read_filter_normalize_scRNA-live.Rmd
02-celltype_assignment-live.Rmd
03-dataset_integration-live.Rmd
04-differential_expression-live.Rmd
data
diagrams
exercise_01-citeseq.Rmd
exercise_02-celltype.Rmd
exercise_03-integration.Rmd
exercise_04-diffexp.Rmd
README.md

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

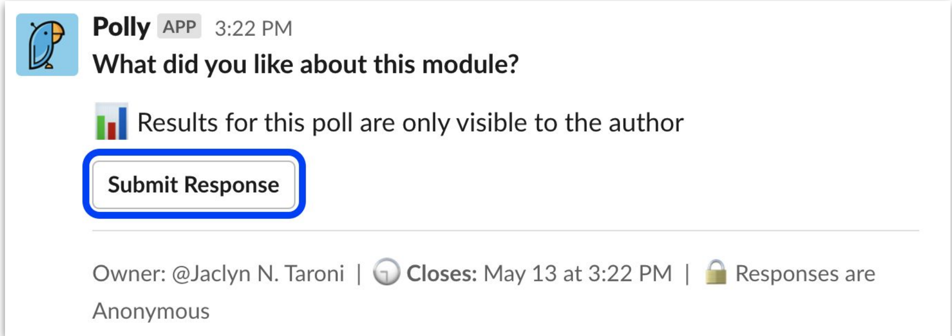


`dplyr`

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`	`%>%`	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`	`select()`	Select	Selects columns that match the specified argument
`dplyr`	`mutate()`	Mutate	Adds a new column that is a function of existing columns
`dplyr`	`summarise()`	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`	`group_by()`	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.

We want your feedback!



Polly APP 3:22 PM

What did you like about this module?

Results for this poll are only visible to the author

Owner: @Jaclyn N. Taroni | **Closes:** May 13 at 3:22 PM | Responses are Anonymous

At the end of each module, we will post a few questions in the Slack channel.

- The most difficult or confusing point of the module ("muddiest point")
  We will post additional material answering your questions the next day
  Responses to this question will appear in the channel anonymously

- What did you like about the module?
- How we can improve the module?
  These responses will be collected anonymously (and not posted)

Friday Afternoon

Your own projects
Exercise notebooks

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

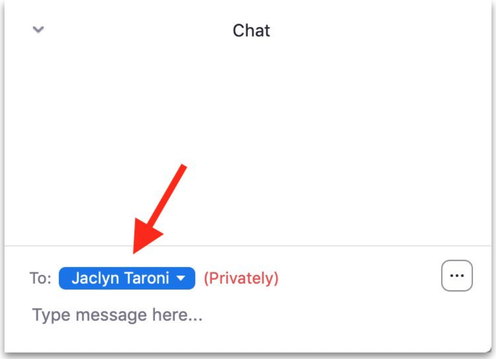
Presentations

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

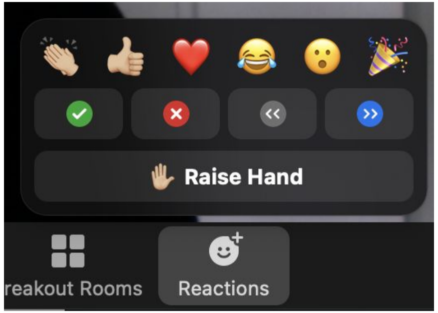
Virtual Training Procedures

General Zoom Etiquette

- Keep your microphone muted
- Type questions in the Chat window (directed to the host)
  - Click on the “Chat” button at the bottom of your window to open the chat.

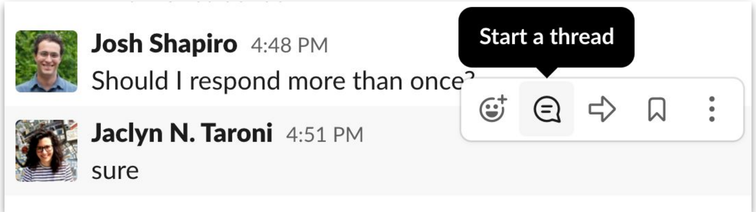


- Use the Zoom reactions to tell us how you are doing!



We encourage you to use Slack

- You have been added to the **#2023-march-training** Slack channel
- Post public questions, get help with errors and debugging, make comments, and help others!
  - Use threads to keep related content together
- Watch out for feedback polls!
- Stay in touch after the workshop!



Josh Shapiro 4:48 PM
Should I respond more than once?

Jaclyn N. Taroni 4:51 PM
sure

Communication during instruction

zoom

- **I have an urgent question** that needs an answer before moving on:
  - *Raise Hand* or *Chat* with the room host
- **I'm stuck with an error** and can't proceed with the hands-on exercise
  - *Chat* with meeting host: Request 1:1 and you will be placed in a breakout room with a Data Lab staff member

slack

- **I have an general question** that does not need an answer right away.
  - *Post* in *#2023-march-training*
- **I'm having trouble logging in** to RStudio Server
  - *Direct Message* a Data Lab staff member (not the current host or instructor)

Trouble logging into Zoom and Slack? ***Email*** training@ccdatalab.org

Communication at other times (consultation periods)

slack

- I have questions about **previous instruction** or **exercise notebooks**
  - ***Post*** in *#2023-march-training*
  - If you need to share your screen, we will set up a 1:1 or group Zoom call

- **I would like to be paired up with other participants**
  - ***Post*** in *#2023-march-training*; we can set you up in a Zoom breakout room

- I have a question that is **highly specific to my data**
  - ***Direct Message*** a Data Lab staff member

- **I'm having trouble logging in** to RStudio Server
  - ***Direct Message*** a Data Lab staff member

Trouble logging into Zoom and Slack? ***Email*** training@ccdatalab.org

ScPCA Downstream Analysis Usability Evaluations

- The Single-cell Pediatric Cancer Atlas (ScPCA) Portal is a database of uniformly processed single-cell data from pediatric cancer clinical samples and xenografts.
- We developed the ScPCA downstream analysis pipeline for use with data available on the Portal. You can filter, normalize, and cluster data with the pipeline.
- We're conducting usability evaluations as a structured way to gather feedback on how the pipeline is currently functioning. During a ~1-hour session you will be asked to use the documentation, run the pipeline, and discuss your experience.
- Interested in participating? Schedule a session with Deepa. Everyone who participates will receive Data Lab swag!