HBC
Harvard Chan Bioinformatics Core

Pseudobulk and related approaches for scRNA-seq analysis

https://tinyurl.com/hbc-pseudobulk-DGE

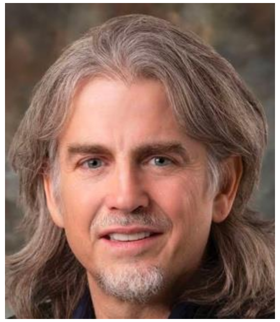
Harvard Chan Bioinformatics Core

Introductions!

Shannan Ho Sui
Director

Meeta Mistry
Associate Director

Lorena Pantano
Director of Bioinformatics Platform



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Faculty Advisor

Upen Bhattarai

Heather Wick

Will Gammerdinger

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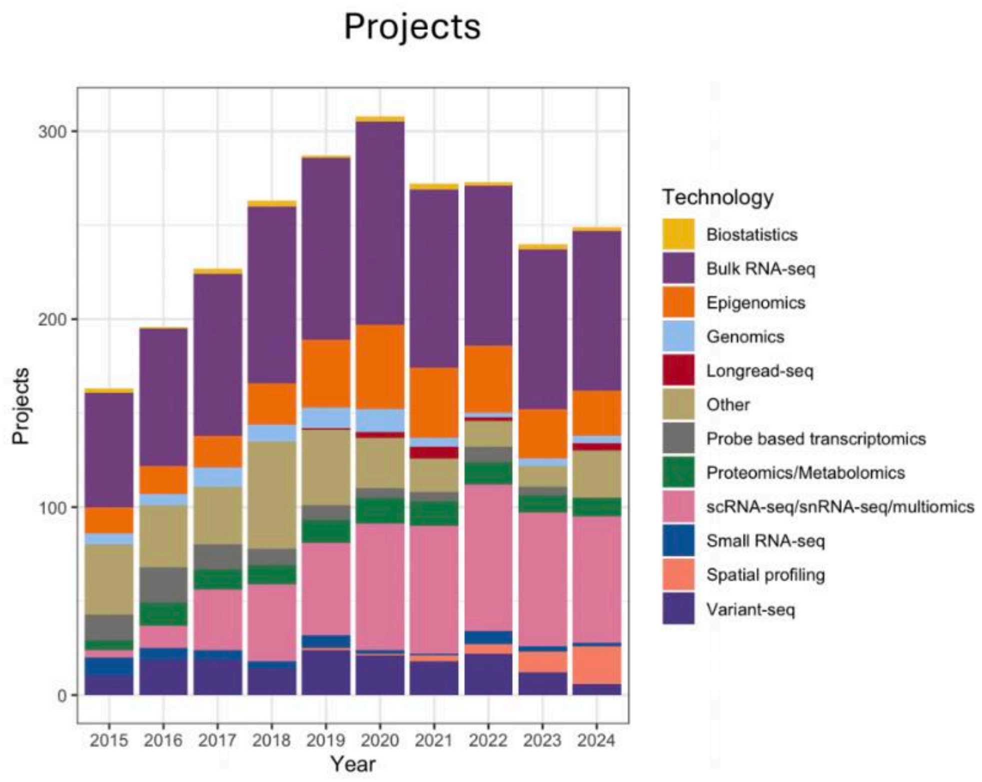
Consulting

- Transcriptomics: Bulk, single cell, small RNA
- Epigenomics: ChIP-seq, CUT&RUN, ATAC-seq, DNA methylation
- Variant discovery: WGS, resequencing, exome-seq and CNV
- Multiomics integration
- Spatial biology
- Experimental design and grant support



https://bioinformatics.sph.harvard.edu/services

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CFAR
HARVARD UNIVERSITY
CENTER FOR AIDS RESEARCH

HARVARD
T.H. CHAN
SCHOOL OF PUBLIC HEALTH

NIEHS

HARVARD
CATALYST

THE HARVARD CLINICAL
AND TRANSLATIONAL
SCIENCE CENTER

HARVARD
MEDICAL SCHOOL

Training

- Hands-on workshops design to reflect best practices, reproducibility and an emphasis on experimental design
  - Basic Data Skills
    - Shell
    - R
  - Advanced Topics: Analysis of high-throughput sequencing data
    - Chromatin Biology
    - Bulk RNA-seq
    - Differential Gene Expression
    - scRNA-seq
    - Variant Calling
  - Current Topics in Bioinformatics

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DF/HCC
DANA-FARBER / HARVARD CANCER CENTER

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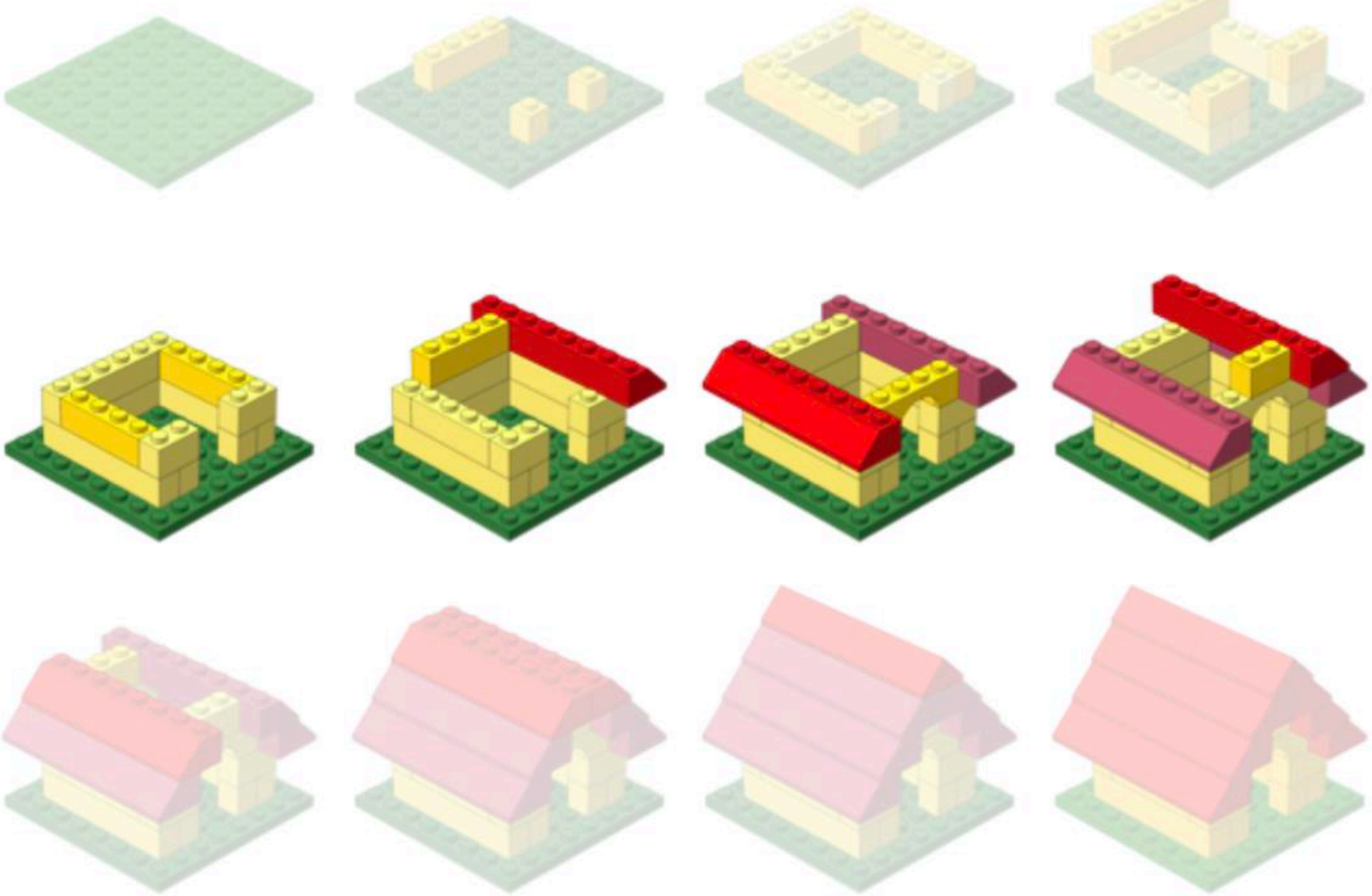
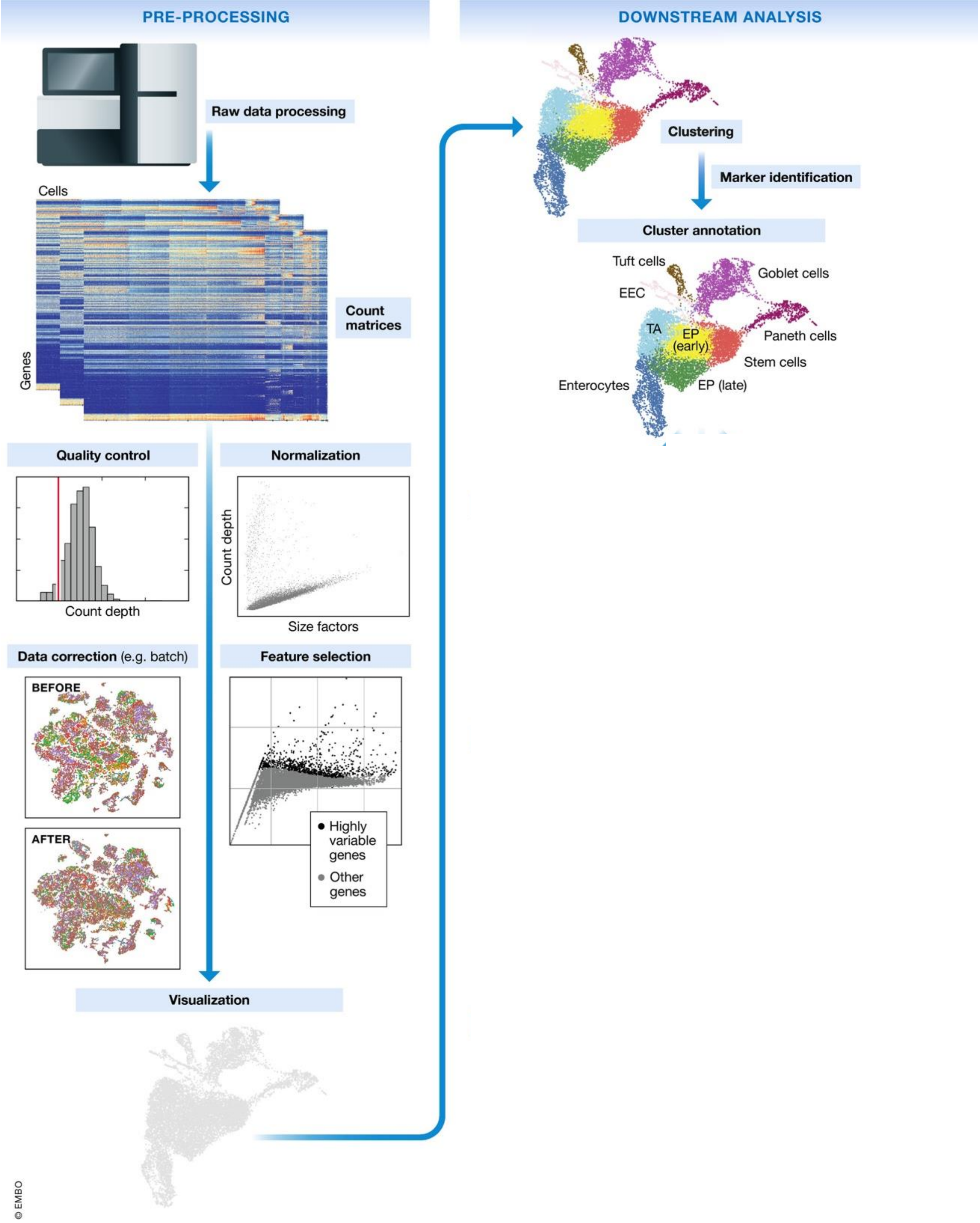
MEDICAL SCHOOL

Workshop scope



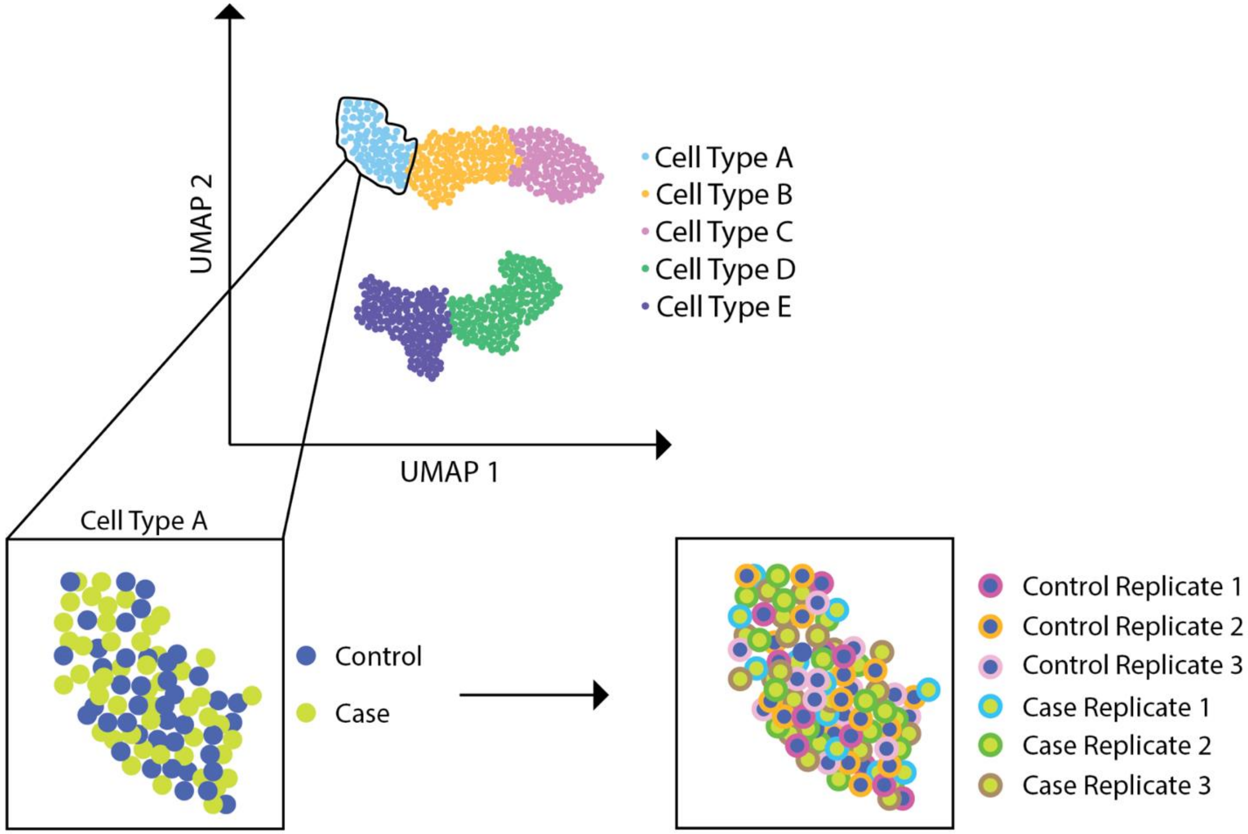
Bioinformatics Data Analysis

What is NOT covered in this workshop

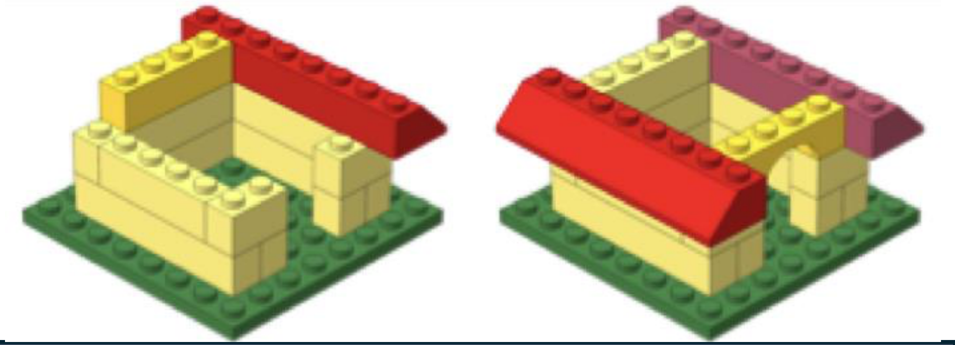


Luecken, MD and Theis, FJ. Current best practices in single-cell RNA-seq analysis: a tutorial, Mol Syst Biol 2019 (doi: https://doi.org/10.15252/msb.20188746)

What IS covered in this workshop



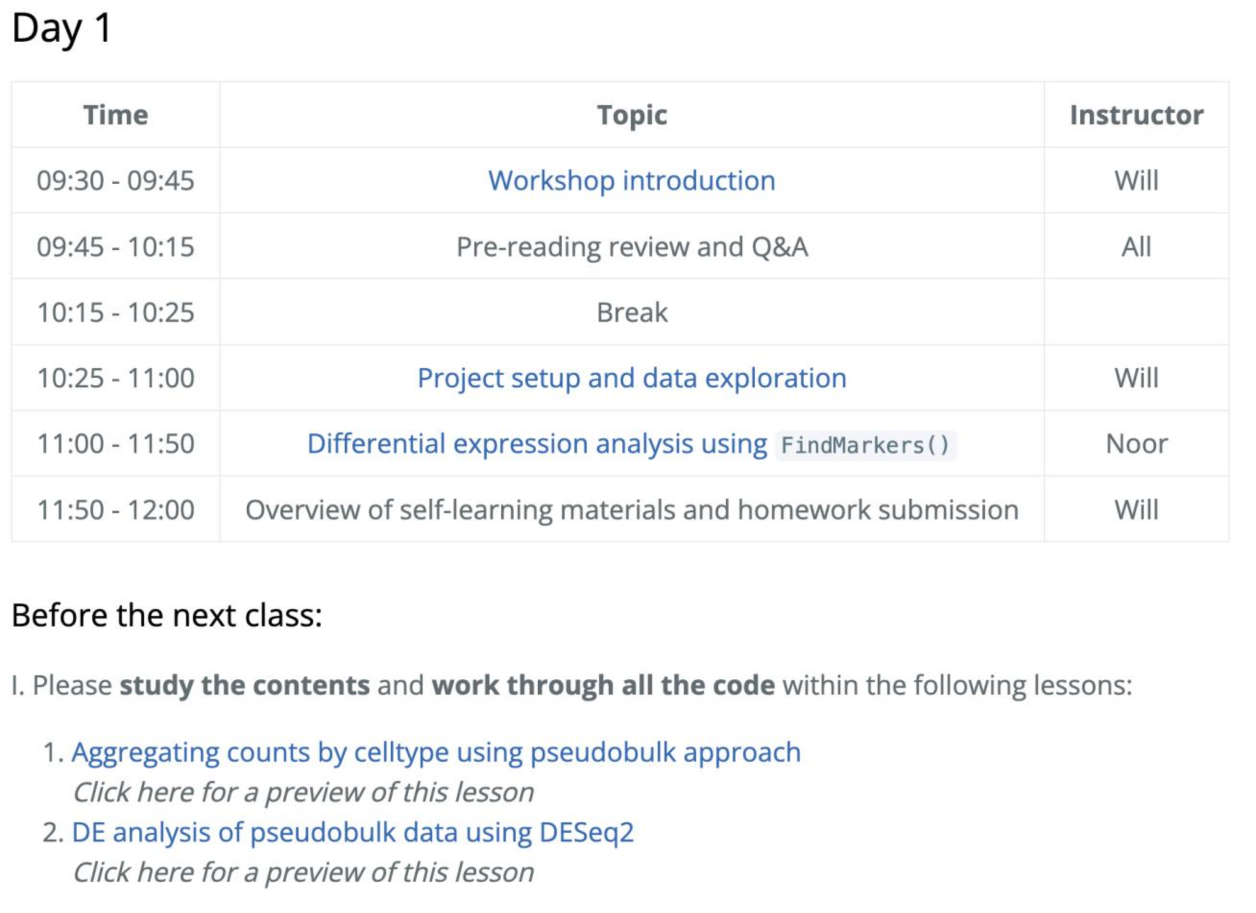
Workshop Scope



- Understanding considerations for when to use different DGE algorithms on scRNA-seq data
- Using FindMarkers to evaluate significantly DE genes
- Aggregating single cell expression data into a pseudobulk counts matrix to run a DESeq2 workflow
- Evaluating expression patterns of differentially expressed genes at the pseudobulk and single cell level
- Application of methods for evaluating differential proportions of cells between conditions

Logistics

Course schedule



Day 1

Time	Topic	Instructor
09:30 - 09:45	Workshop introduction	Will
09:45 - 10:15	Pre-reading review and Q&A	All
10:15 - 10:25	Break	
10:25 - 11:00	Project setup and data exploration	Will
11:00 - 11:50	Differential expression analysis using `FindMarkers()`	Noor
11:50 - 12:00	Overview of self-learning materials and homework submission	Will

Before the next class:

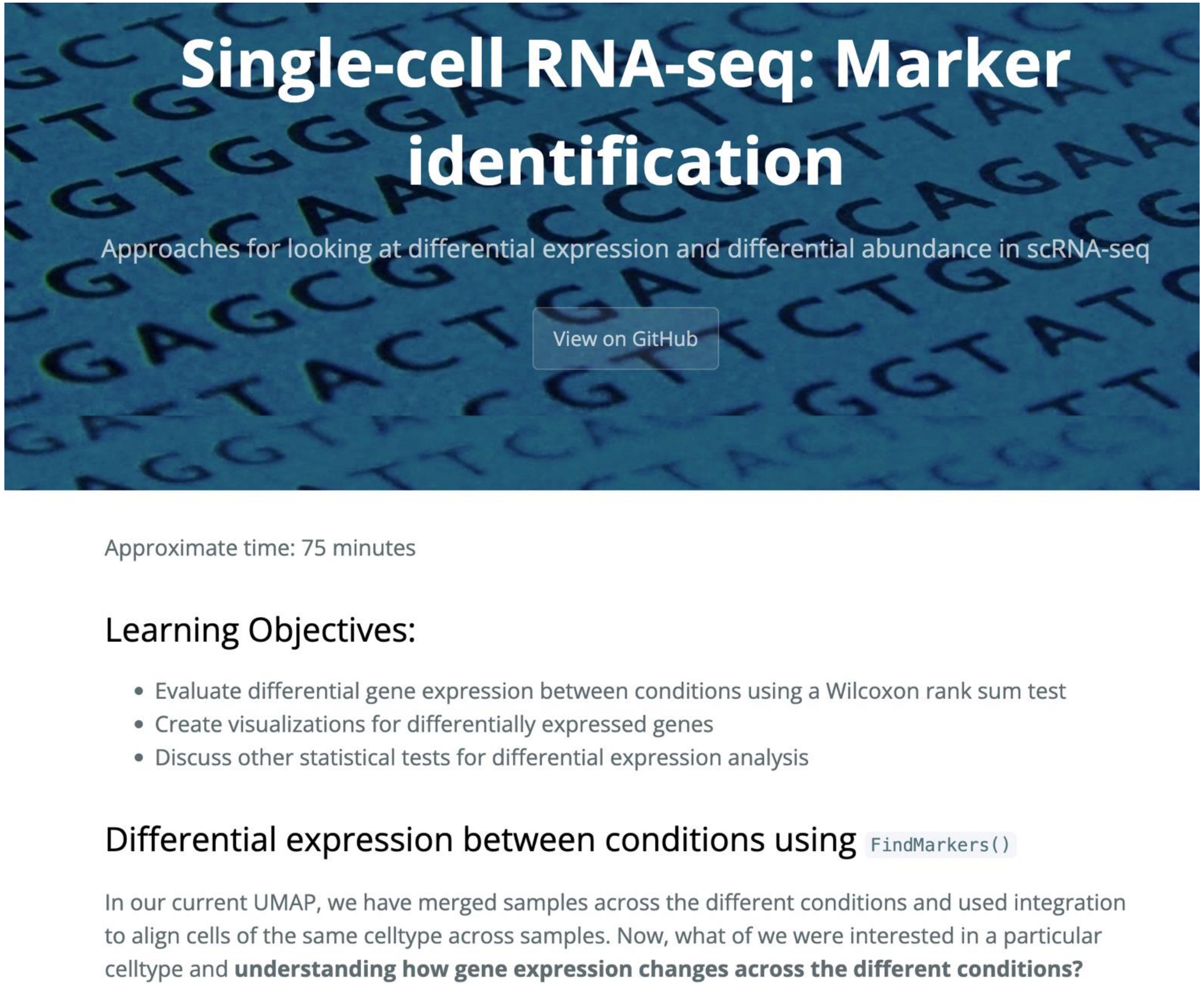
I. Please **study the contents** and **work through all the code** within the following lessons:

1. Aggregating counts by celltype using pseudobulk approach
   Click here for a preview of this lesson
2. DE analysis of pseudobulk data using DESeq2
   Click here for a preview of this lesson

https://tinyurl.com/hbc-pseudobulk-DGE

Course materials

- We continuously update our materials to reflect changes in the field/software



Single-cell RNA-seq: Marker identification

Approaches for looking at differential expression and differential abundance in scRNA-seq

Approximate time: 75 minutes

Learning Objectives:

- Evaluate differential gene expression between conditions using a Wilcoxon rank sum test
- Create visualizations for differentially expressed genes
- Discuss other statistical tests for differential expression analysis

Differential expression between conditions using `FindMarkers()`

In our current UMAP, we have merged samples across the different conditions and used integration to align cells of the same celltype across samples. Now, what of we were interested in a particular celltype and **understanding how gene expression changes across the different conditions?**

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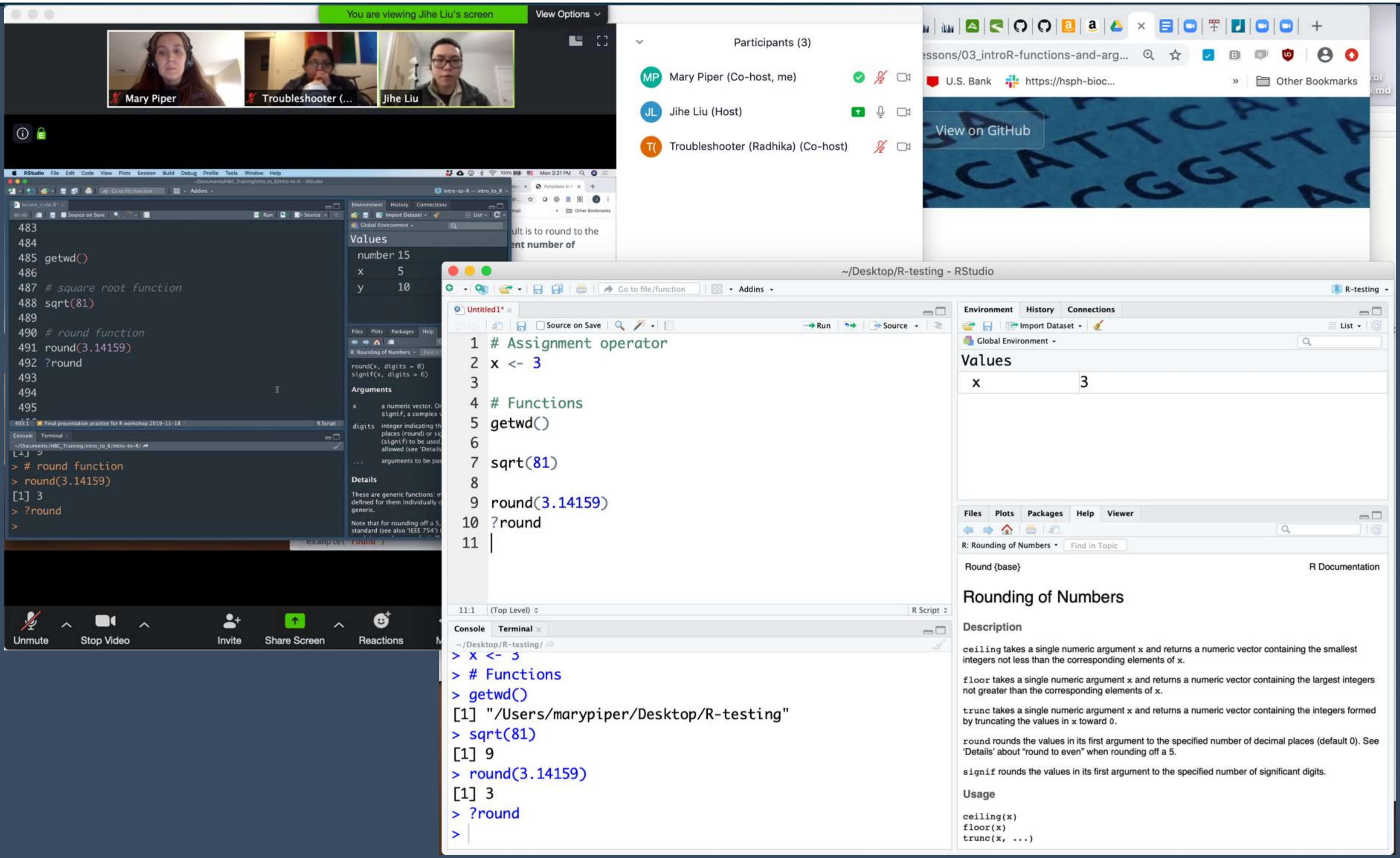
Single Screen & 3 Windows



Participants (3)

- Mary Piper (Co-host, me)
- Jihe Liu (Host)
- Troubleshooter (Radhika) (Co-host)

~/Documents/HBC_Training/intro_to_R/intro-to-R - RStudio

```
getwd()

# square root function
sqrt(81)

# round function
round(3.14159)
?round
```

Final presentation practice for R workshop 2019-11-18

```
> # round function
> round(3.14159)
[1] 3
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Values	
number	15
x	5
y	10

~/Desktop/R-testing - RStudio

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~/Desktop/R-testing/

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R: Rounding of Numbers

Round {base} R Documentation

Rounding of Numbers

Description

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Usage

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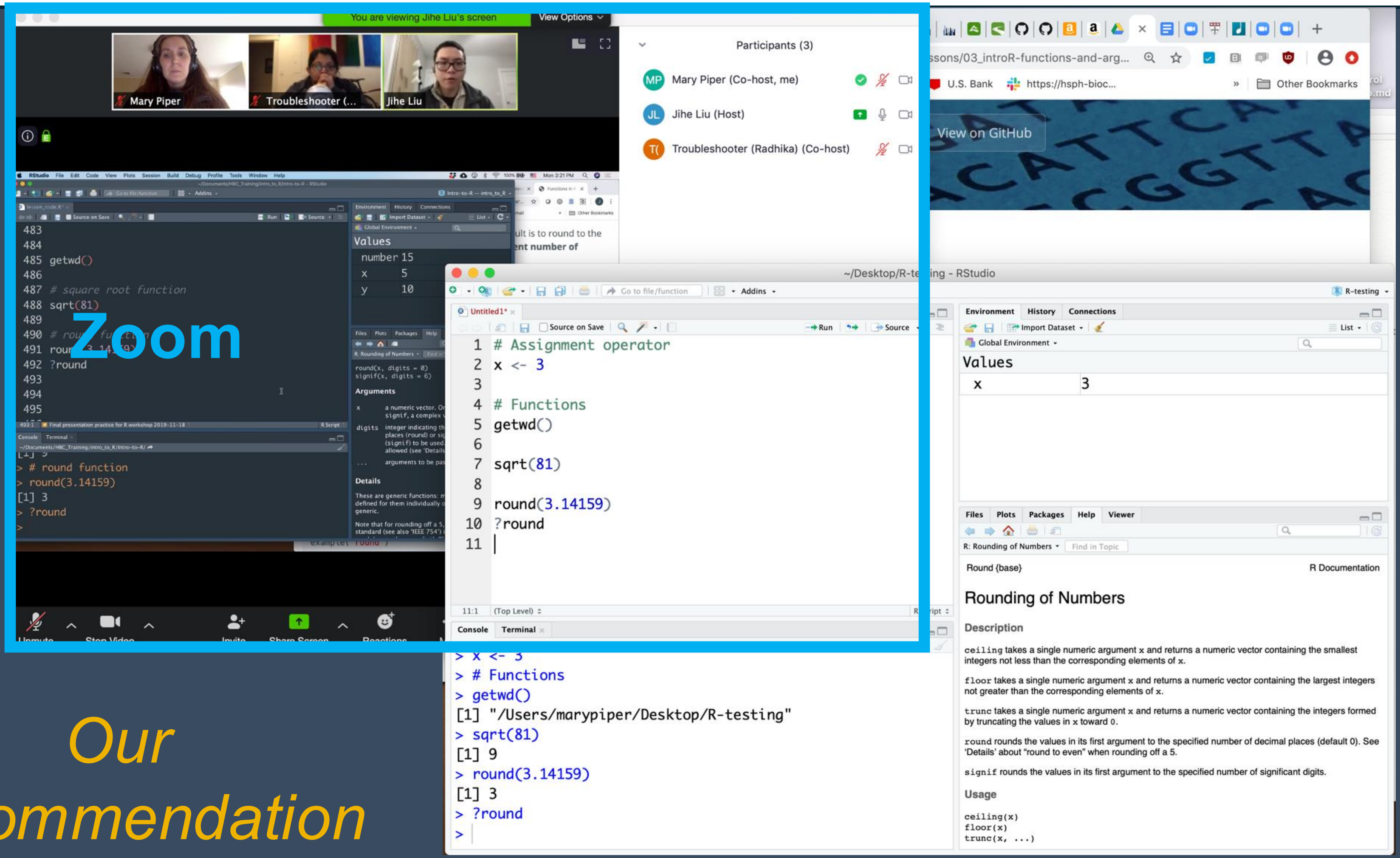
Single Screen & 3 Windows



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Our Recommendation

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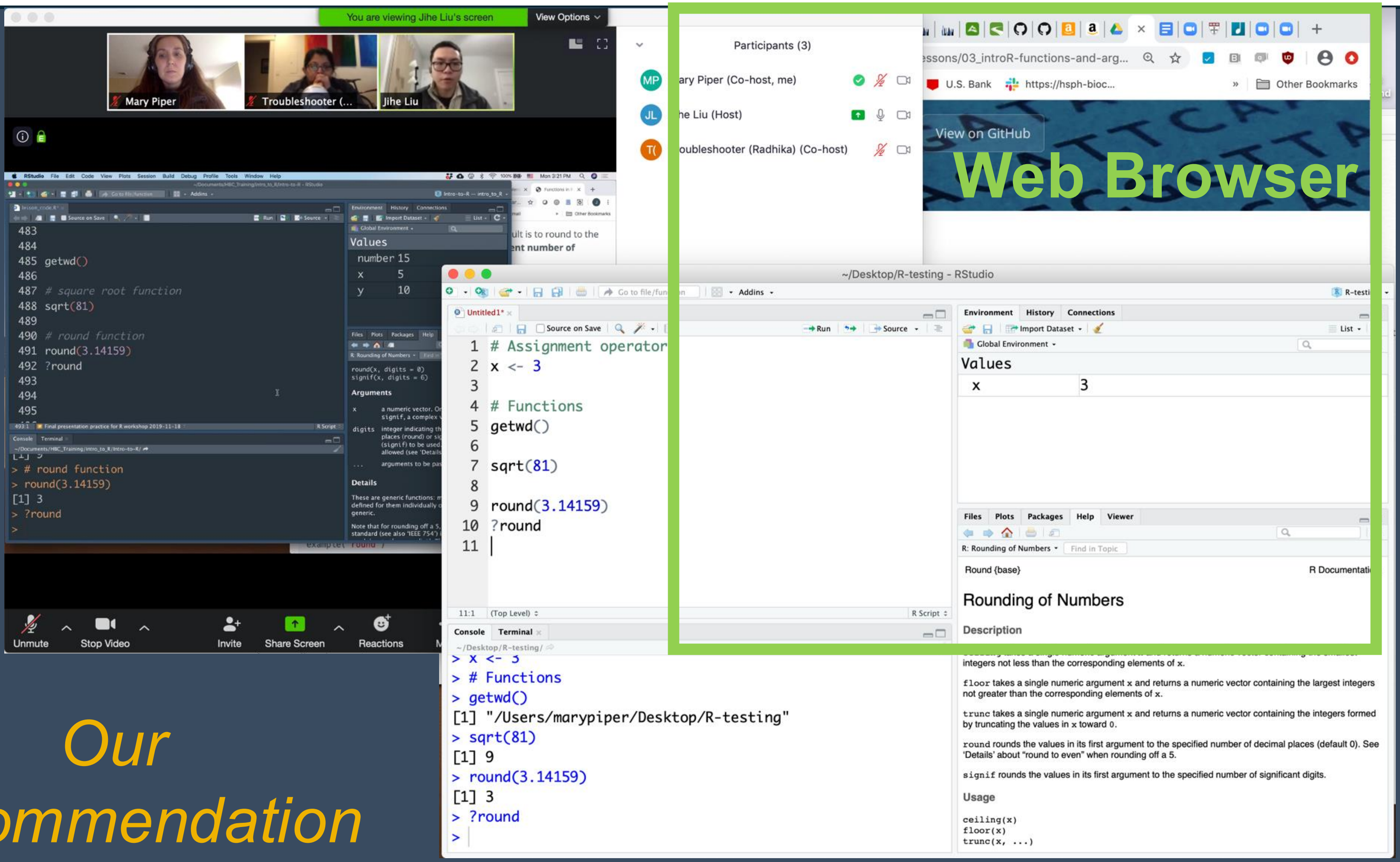

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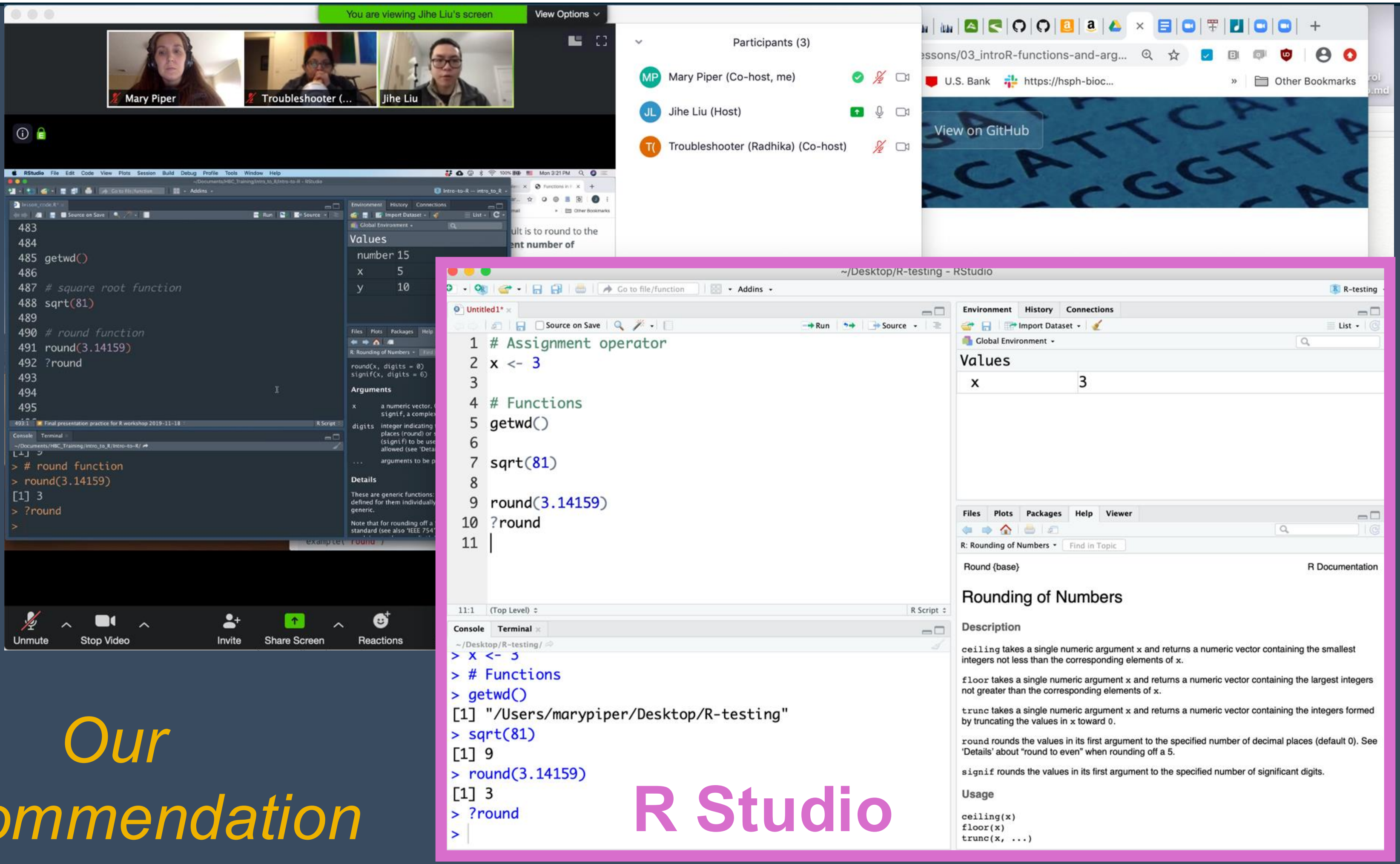

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R Studio

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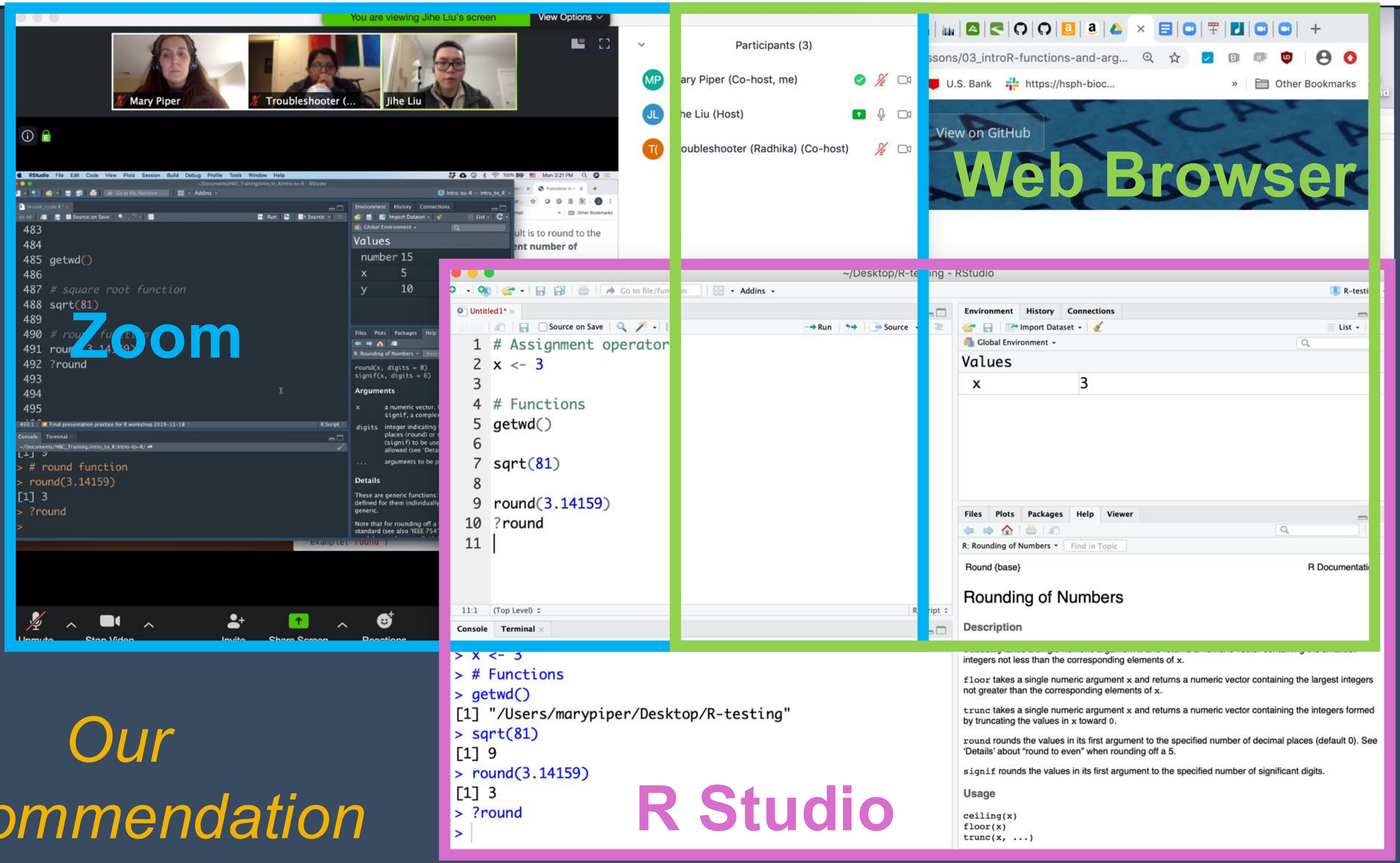

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Our Recommendation

Course participation

- Mandatory review of self-learning lessons and assignments
- Attendance required for all classes
- Your questions and active participation drive learning
- **We look forward to all of your questions!**

Course participation

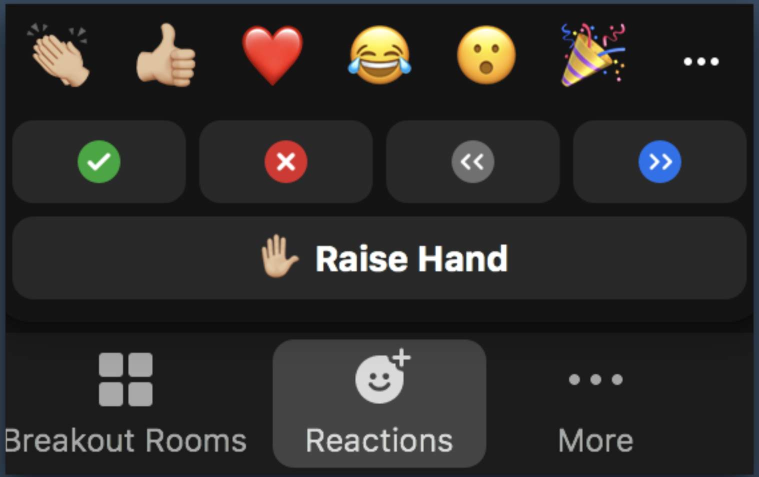
- At-home lessons and exercises after each session
- Cover material not previously discussed
- Provides us feedback to help pace the course appropriately
- 3-5 hours to complete
- Homework load is heavier in the beginning of this workshop series and tapers off

Using AI for Assignments

- Do
    - Try to resolve error messages with it
    - Test code written by AI on a dataset where you have expected results
    - Take the time to review the generated code line-by-line
- Don't
    - Implement it in replacement to learning
    - Write code that you don't understand
    - Assume the output from an AI process is correct

Odds & Ends

- Quit/minimize all applications that are not required for class
- Are you all set?
  - ✅ = "agree", "I'm all set"
  - ❌ = "disagree", "I need help"



Odds & Ends

- Questions for the presenter?
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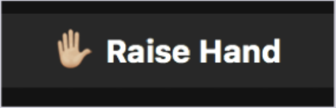
  -  when the presenter asks for questions
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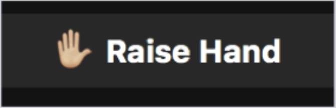
  -  when the presenter asks for questions
  - Let the Moderator know
- Technical difficulties with software?
  - Start a private chat with the Troubleshooter with a description of the problem

Contact Us

- *HBC training team:* hbctraining@hsph.harvard.edu
- *HBC consulting:* bioinformatics@hsph.harvard.edu