



```
dds <- DESeqDataSetFromMatrix(countData = cts,
                                colData = coldata,
                                design= ~ batch + condition)

dds <- DESeq(dds)
resultsNames(dds) # lists the coefficients
res <- results(dds, name="condition_trt_vs_untrt")
# or to shrink log fold changes association with condition:
res <- lfcShrink(dds, coef="condition_trt_vs_untrt", type="apeglm")
```

Bulk RNA-seq Analysis Part II

Differential Gene Expression

Harvard Chan Bioinformatics Core

<https://tinyurl.com/hbc-dge-online>



Shannan Ho Sui
Director



Victor Barrera



Amelie Jule



Zhu Zhuo



Radhika Khetani
Director of Education



Meeta Mistry



Jihe Liu



Will Gammerdinger



Maria Simoneau



James Billingsley



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Sergey Naumenko



Peter Kraft
Faculty Advisor

Consulting

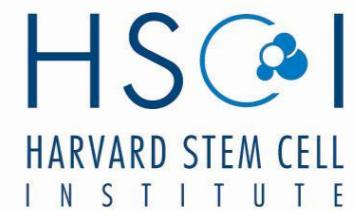
- RNA-seq analysis: bulk, single cell, small RNA
- ChIP-seq and ATAC-seq analysis
- Genome-wide methylation
- WGS, resequencing, exome-seq and CNV studies
- QC & analysis of gene expression arrays
- Functional enrichment analysis
- Grant support

<http://bioinformatics.sph.harvard.edu/>



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NIEHS



Training

1. Basic Data Skills - No prior programming knowledge needed (no prerequisites)
2. Advanced Topics: Analysis of high-throughput sequencing (NGS) data - Certain “Basic” workshops are prerequisites.
3. Current Topics in Bioinformatics Series - Free, monthly, 3-hour workshops (often hands-on)

<http://bioinformatics.sph.harvard.edu/training/>

<https://hbctraining.github.io/main/>



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INSTITUTE

 **HARVARD
CATALYST**
THE HARVARD CLINICAL
AND TRANSLATIONAL
SCIENCE CENTER

 **HARVARD
MEDICAL SCHOOL**

Training

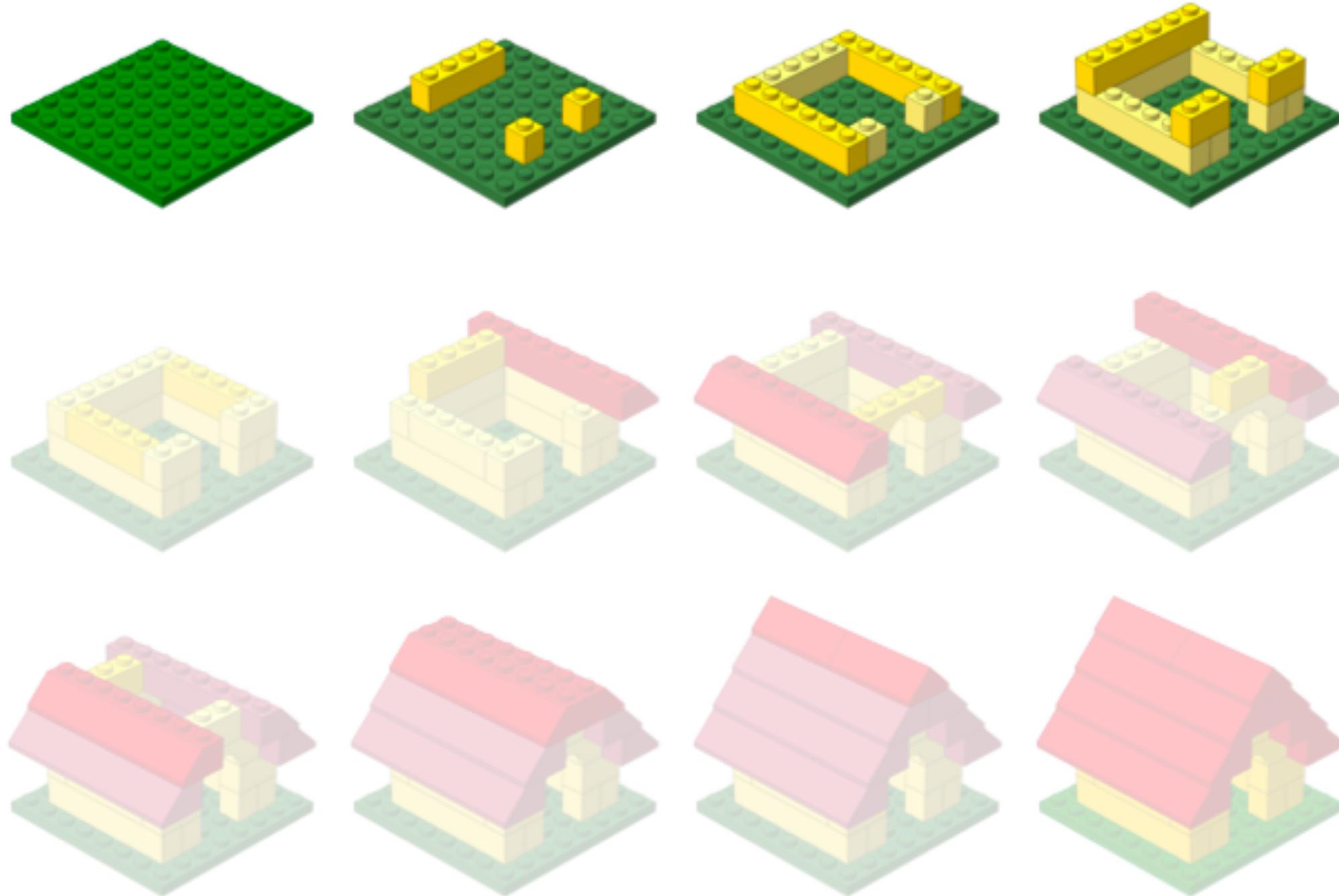
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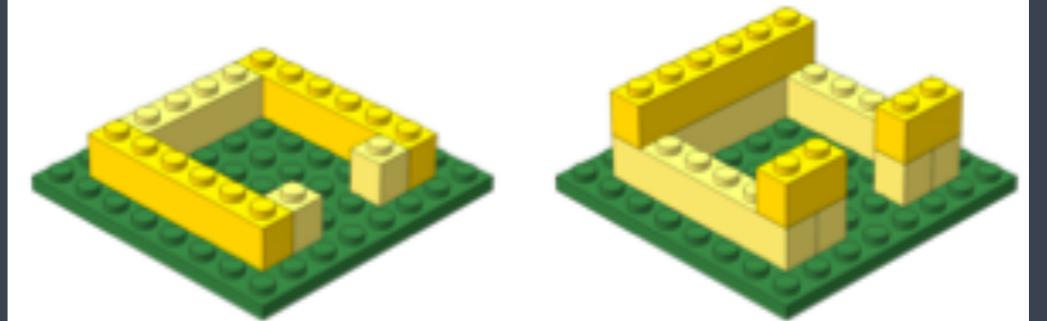
Workshop Scope...



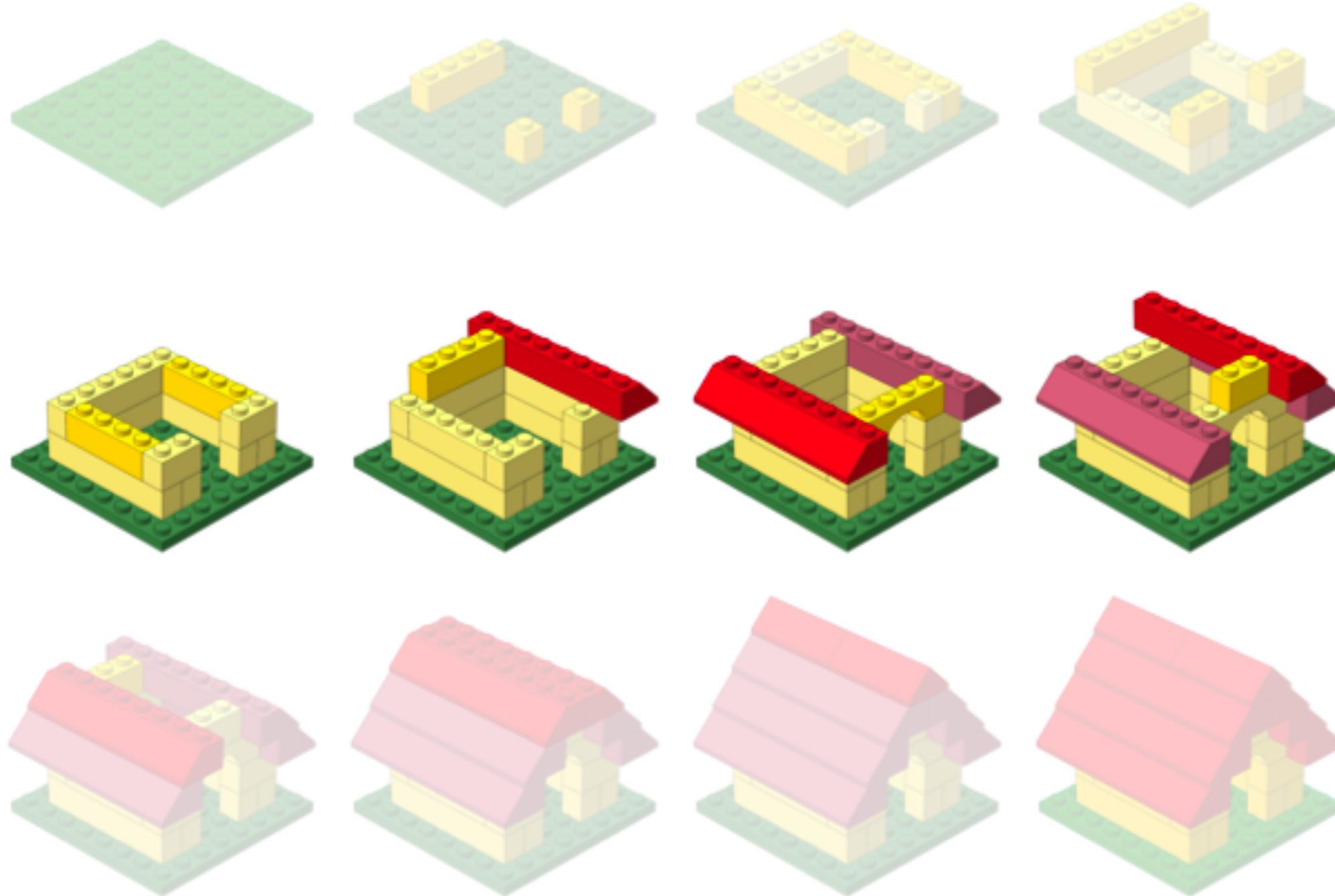
<http://anoved.net/tag/lego/page/3/>

Setting up to perform Bioinformatics analysis

Setting up...



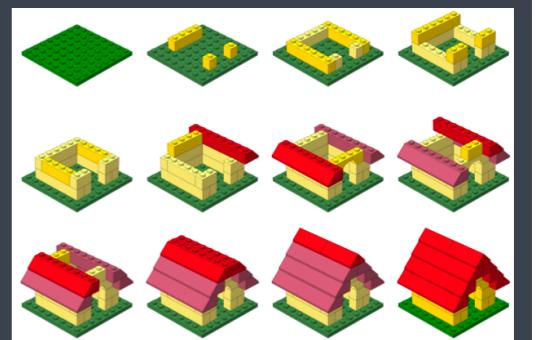
- ✓ Introduction to the command-line interface (shell, Unix, Linux)
 - Dealing with large data files
 - Performing bioinformatics analysis
 - Using tools
 - Accessing and using compute clusters
- ✓ R
 - Parsing and working with smaller results text files
 - Statistical analysis, e.g. differential expression analysis
 - Generating figures from complex data



<http://anoved.net/tag/lego/page/3/>

Bioinformatics data analysis

Workshop Scope



Differential Gene Expression analysis

- ✓ Understand the considerations for performing statistical analysis on RNA-seq data
- ✓ Start with gene counts (after alignment and counting)
- ✓ Perform QC on count data
- ✓ Use DESeq2 to perform differential expression analysis on the count data and obtain a list of significantly different genes
- ✓ Visualize results of the analysis
- ✓ Perform functional analysis on the lists of differentially expressed genes

Logistics

Course webpage

<https://tinyurl.com/hbc-dge-online>

Course schedule online

Workshop Schedule

Pre-reading

1. [Workflow \(raw data to counts\)](#)
2. [Experimental design considerations](#)

Day 1

| Time | Topic | Instructor |
|---------------|--|------------|
| 10:00 - 10:30 | Workshop Introduction | Jihe |
| 10:30 - 10:45 | R refresher Q & A | Radhika |
| 10:45 - 11:15 | RNA-seq pre-reading discussion | Radhika |
| 11:15 - 12:00 | Intro to DGE / setting up DGE analysis | Meeta |

Before the next class:

1. Please **study the contents** and **work through all the code** within the following lessons:
 - [RNA-seq counts distribution](#)
 - [Count normalization](#)
 - [Sample-level QC \(PCA and hierarchical clustering\)](#)
2. **Complete the exercises:**
 - Each lesson above contain exercises; please go through each of them.
 - **Copy over** your code from the exercises into a text file.
 - **Upload the saved text file to Dropbox** the **day before the next class**.

Questions?

- **If you get stuck due to an error** while running code in the lesson, [email us](#)
- Post any **conceptual questions** that you would like to have **reviewed in class here**.

Course webpage

Introduction to DGE

[View on GitHub](#)

Approximate time: 60 minutes

Learning Objectives

- Explore different types of normalization methods
- Become familiar with the `DESeqDataSet` object
- Understand how to normalize counts using DESeq2

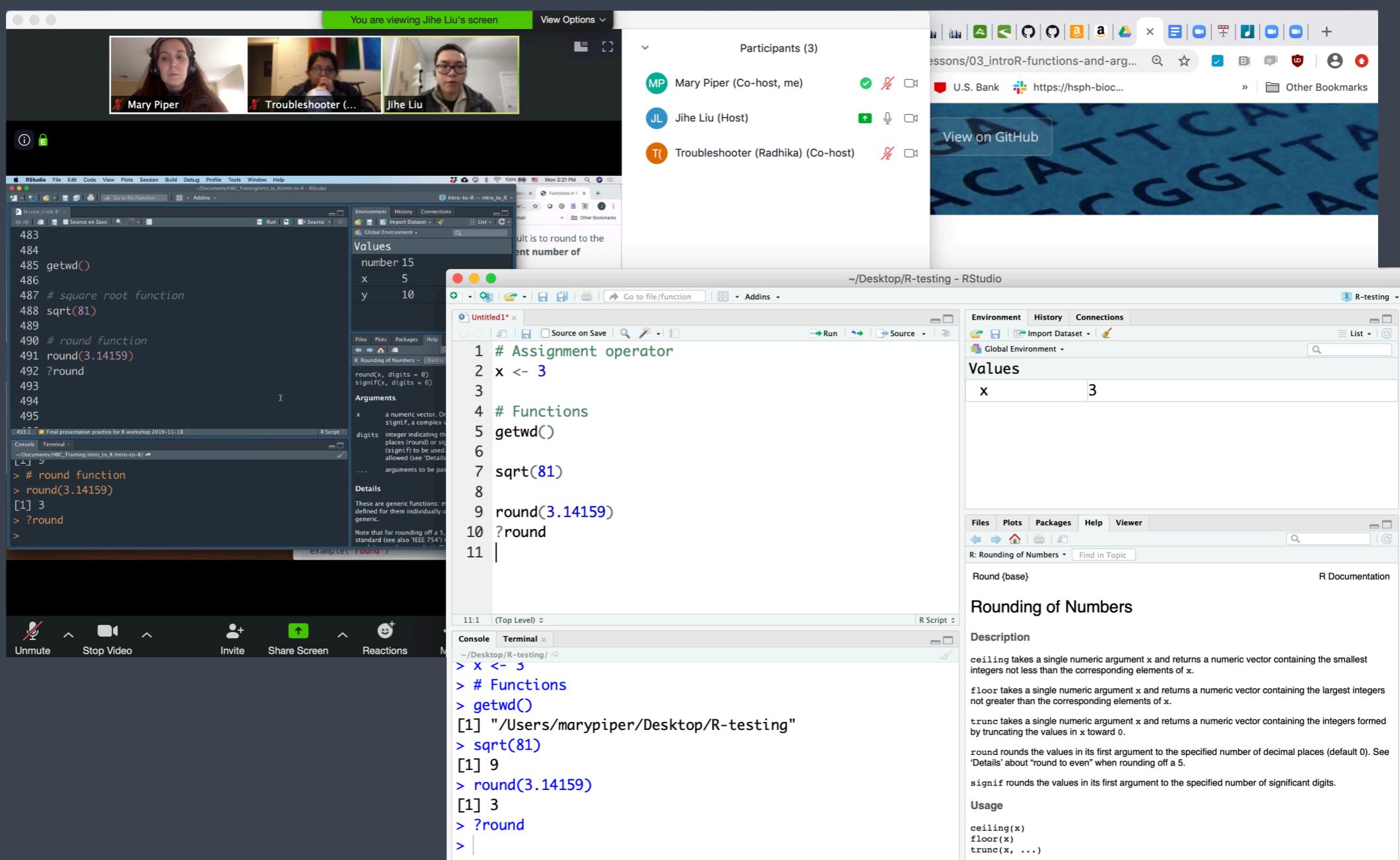
Normalization

The first step in the DE analysis workflow is count normalization, which is necessary to make accurate comparisons of gene expression between samples.

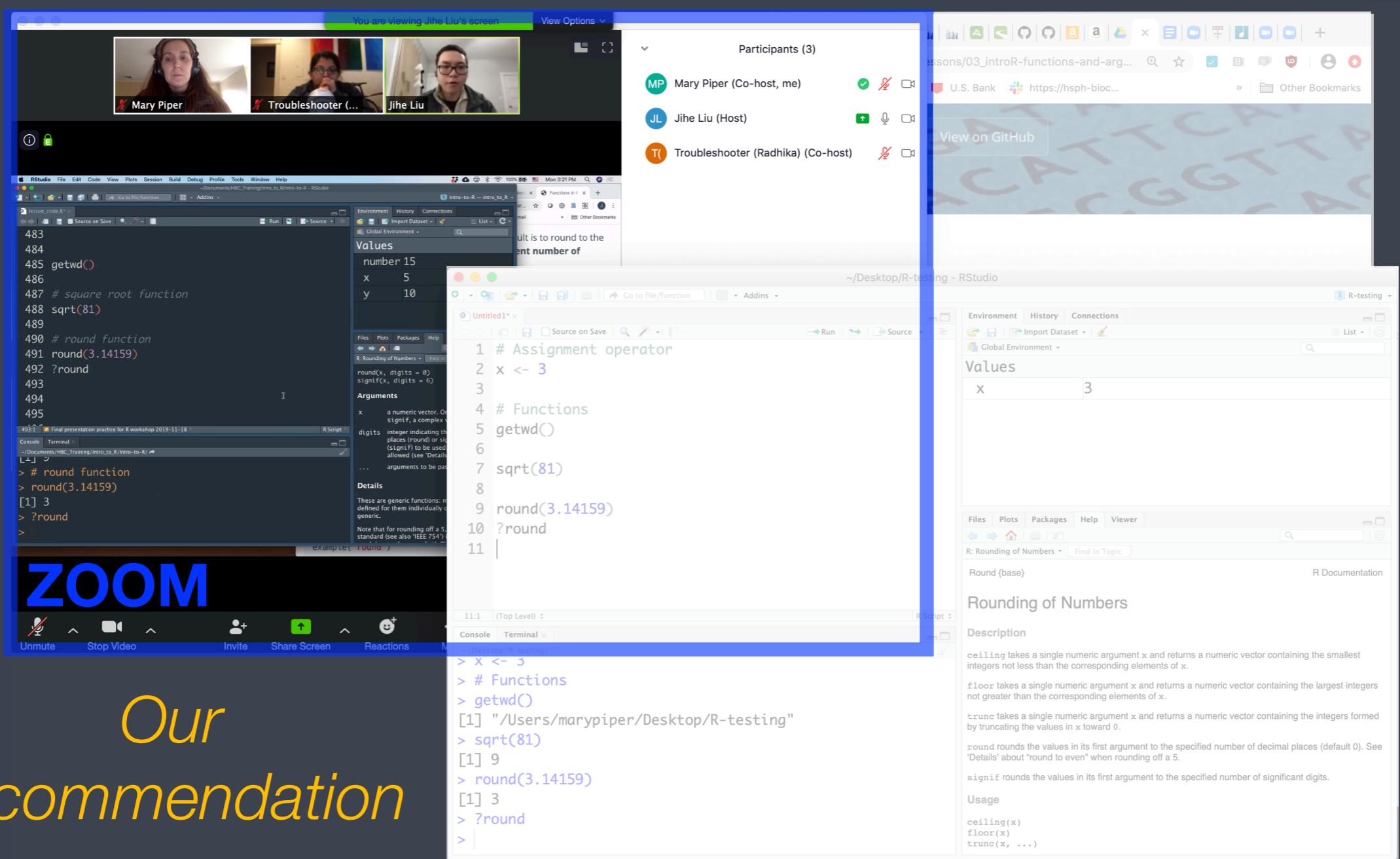
```
graph TD; A["Pseudocounts with  
Kallisto, Sailfish, Salmon"] --> B["Read counts  
associated with genes"]; B --> C["Normalization"]; C --> D["Unsupervised clustering analyses"]; C -.-> E["Quality control"]
```

The flowchart illustrates the DE analysis workflow. It begins with 'Pseudocounts with Kallisto, Sailfish, Salmon', followed by 'Read counts associated with genes'. This leads to 'Normalization', which then leads to 'Unsupervised clustering analyses'. A bracket on the right side groups 'Normalization' and 'Unsupervised clustering analyses' under the heading 'Quality control'.

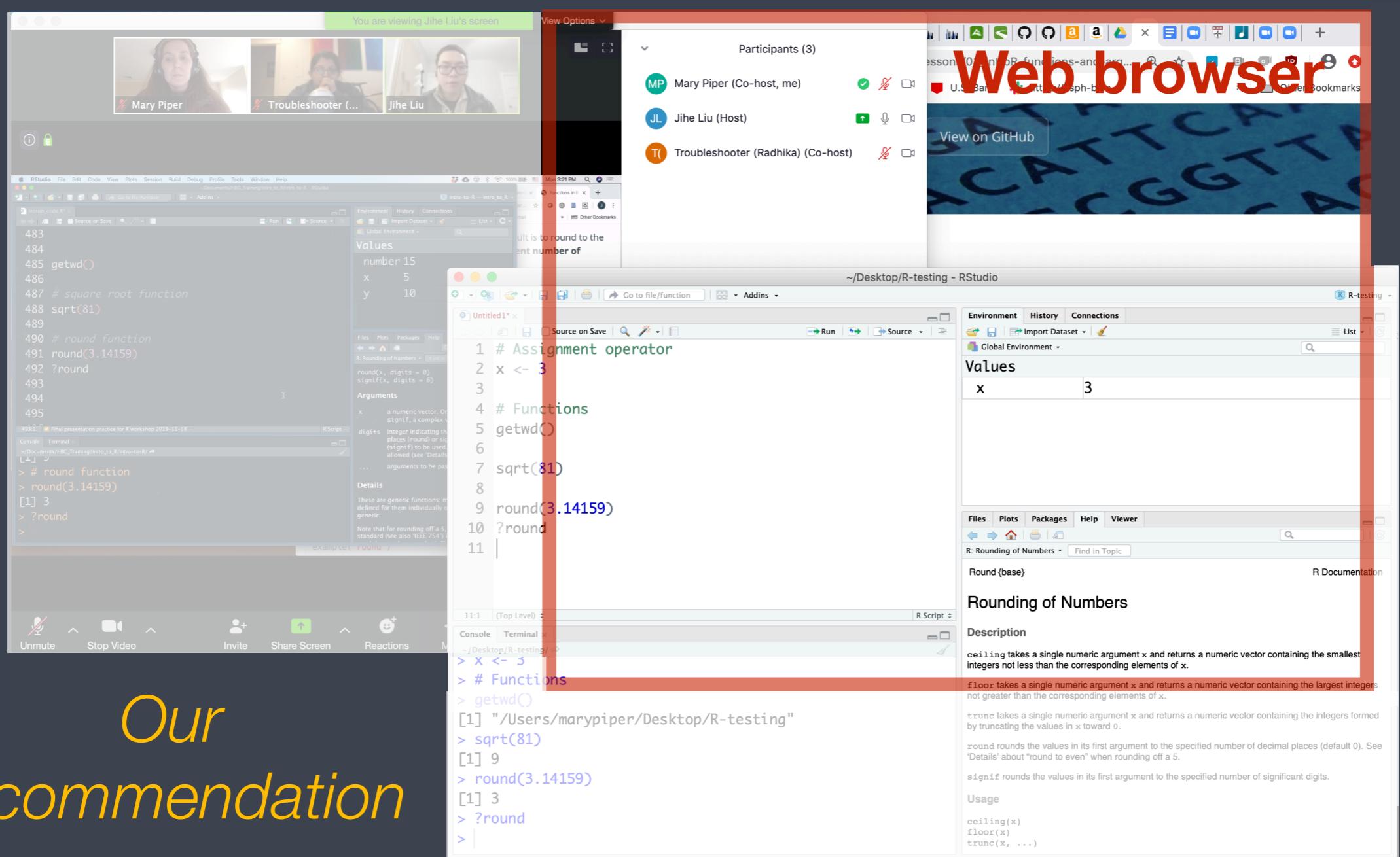
Single screen & 3 windows?



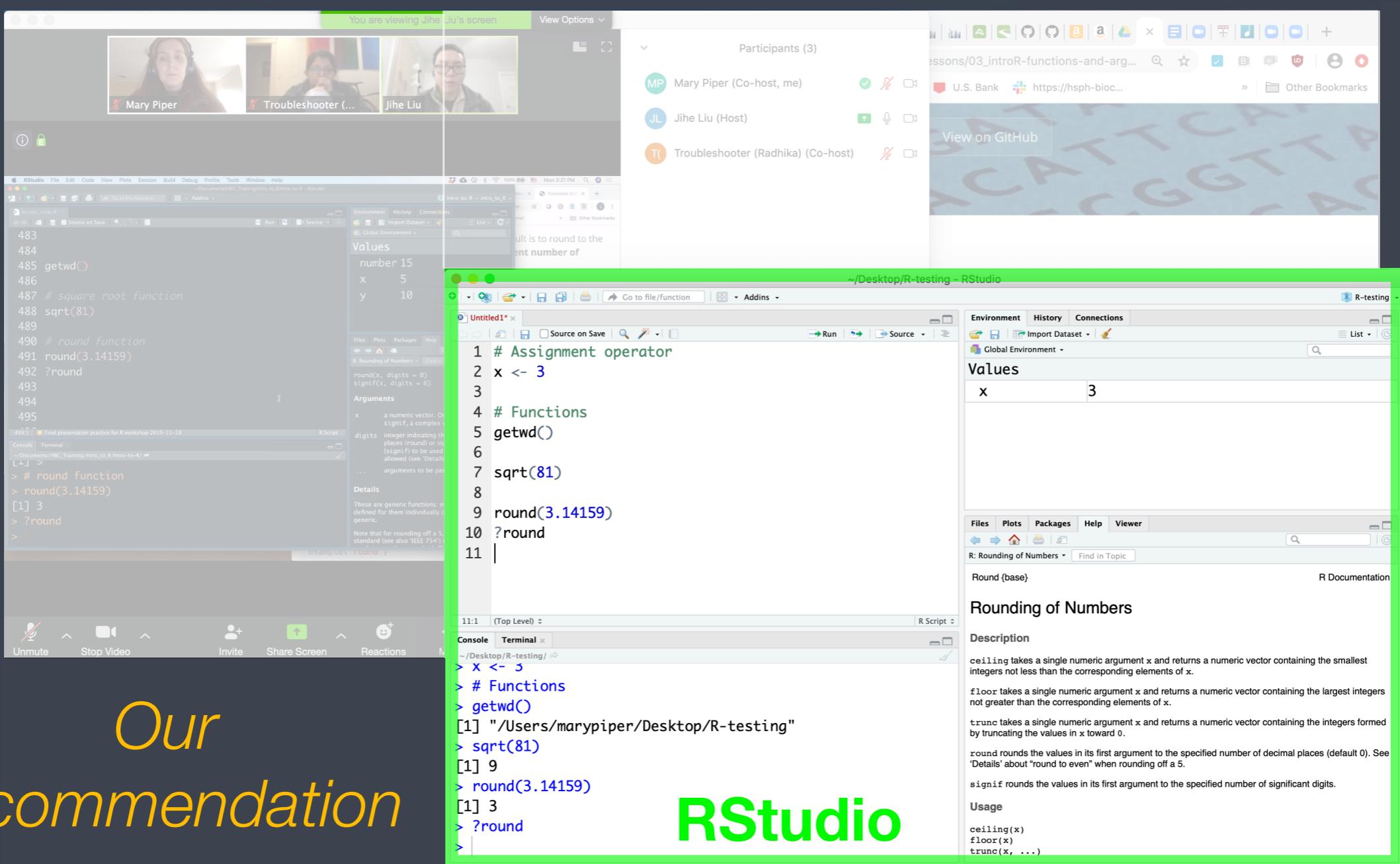
Single screen & 3 windows?



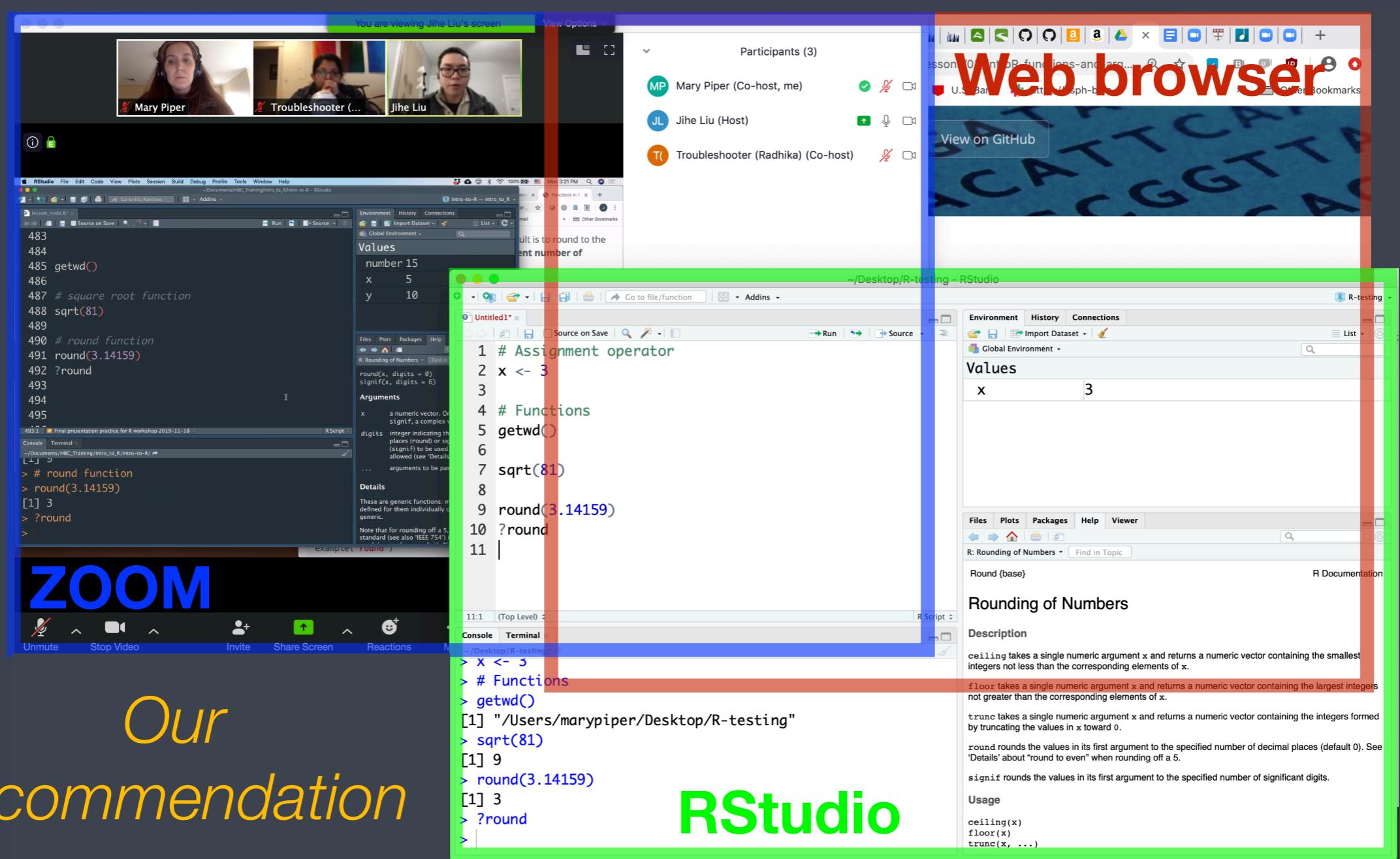
Single screen & 3 windows?



Single screen & 3 windows?



Single screen & 3 windows?

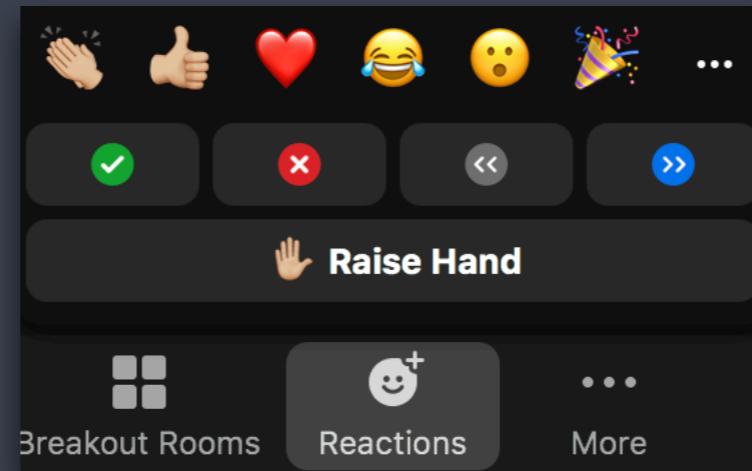


Odds and Ends

- ❖ Quit/minimize all applications that are not required for class
- ❖ Captioning is available upon request

Odds and Ends (1/2)

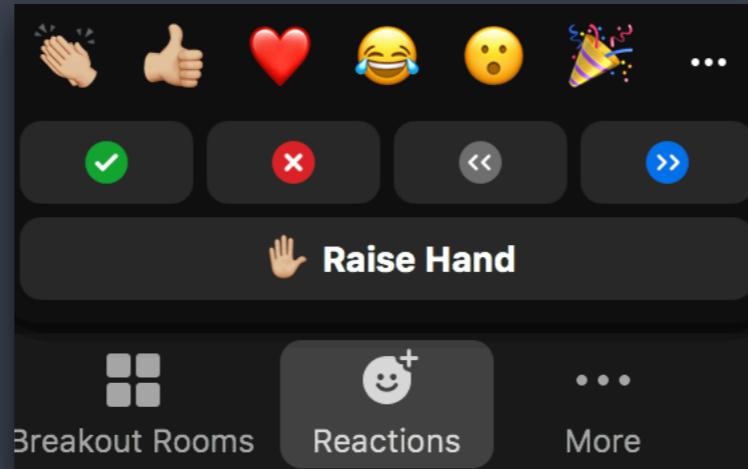
- ❖ Quit/minimize all applications that are not required for class
- ❖ Captioning is available upon request
- ❖ Are you all set?
 - ▶  = "agree", "I'm all set" (equivalent to a **green post-it**)
 - ▶  = "disagree", "I need help" (equivalent to a **red post-it**)



Odds and Ends (2/2)

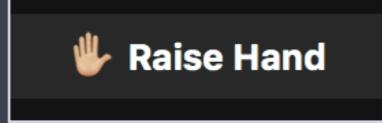
- ❖ Questions for the presenter?

- Post the question in the Chat window OR
-  when the presenter asks for questions
- Let the Moderator know



Odds and Ends (2/2)

- ❖ Questions for the presenter?

- Post the question in the Chat window OR
-  when the presenter asks for questions
- Let the Moderator know

- ❖ Technical difficulties with RStudio or R?

- 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

Twitter

[@bioinfocore](https://twitter.com/bioinfocore)