



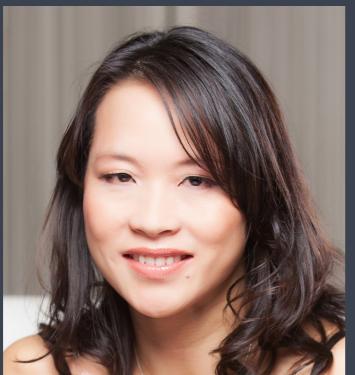
```
dds <- DESeqDataSetFromMatrix(countData = cts,
                                colData = coldata,
                                design= ~ batch + condition)

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")
```

Introduction to Differential Gene Expression Analysis

Harvard Chan Bioinformatics Core

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



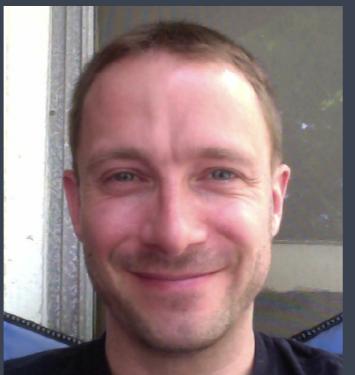
Shannan Ho Sui
Director



John Hutchinson
Associate Director



Victor Barrera



Rory Kirchner



Zhu Zhuo



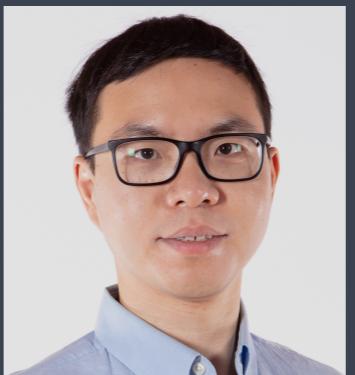
Preetida Bhetariya



Meeta Mistry



Mary Piper



Jihe Liu



Radhika Khetani
Training Director



Ilya Sytchev



James Billingsley



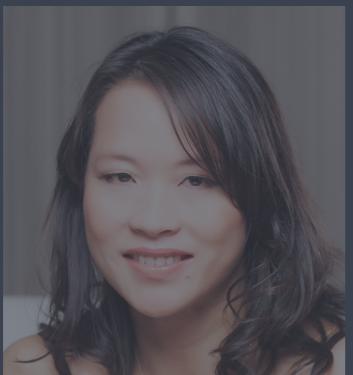
Sergey Naumenko



Joon Yoon



Peter Kraft
Faculty Advisor



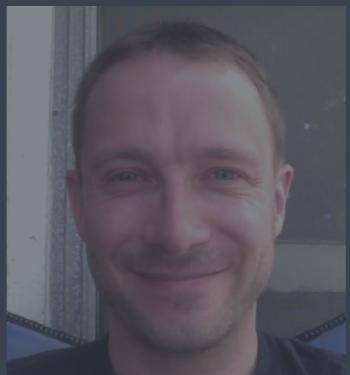
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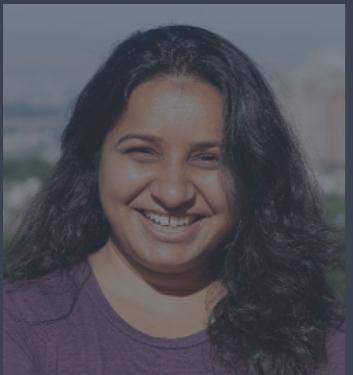
Victor Barrera



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Zhu Zhuo



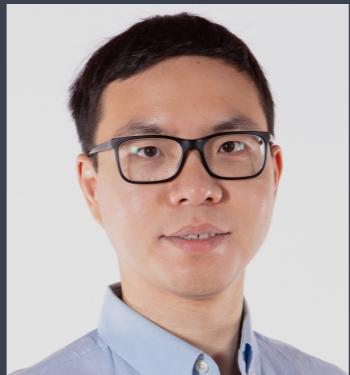
Preetida Bhetariya



Meeta Mistry



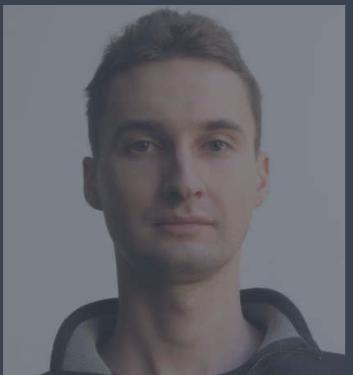
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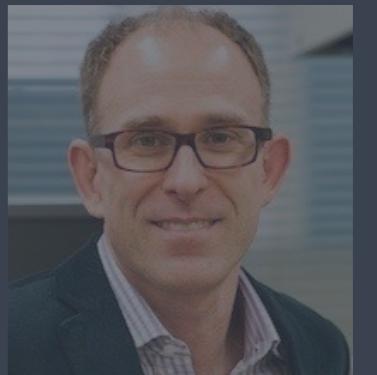
James Billingsley



Sergey Naumenko



Joon Yoon



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/>



**HARVARD
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Training

We have divided our short workshops into 2 categories:

1. Basic Data Skills - No prior programming knowledge needed (no prerequisites)
2. Advanced Topics: Analysis of high-throughput sequencing (NGS) data - Certain “Basic” workshops required as prerequisites.

Any participants wanting to take an advanced workshop will have to have taken the appropriate basic workshop(s) within the past 6 months.

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

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



Training

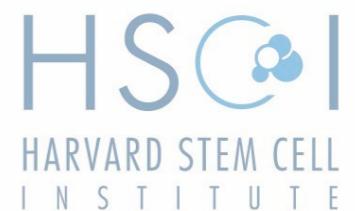
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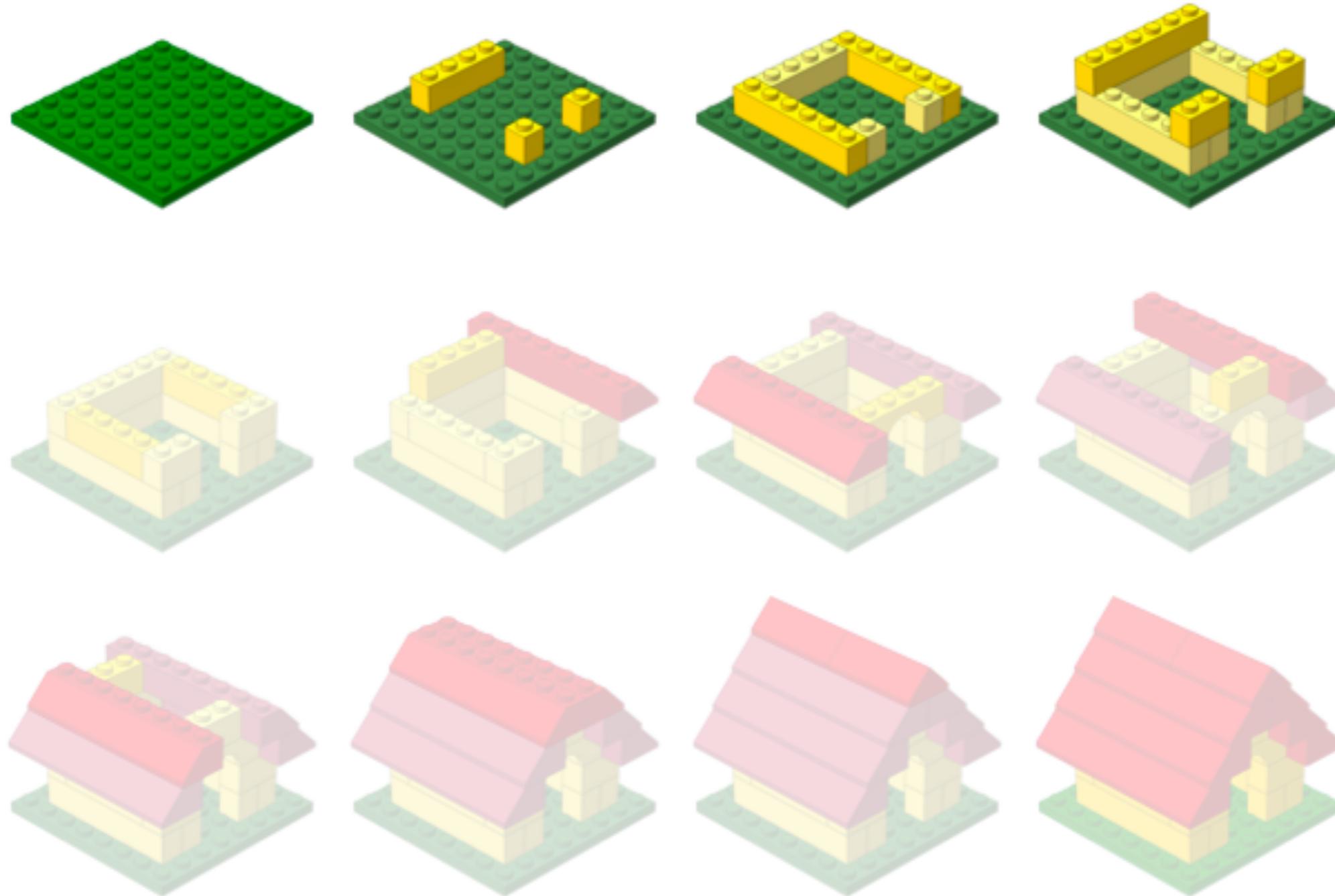
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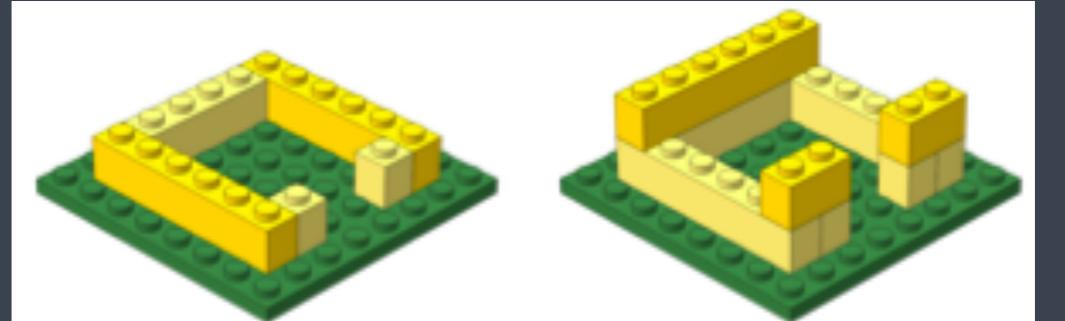
Workshop Scope...



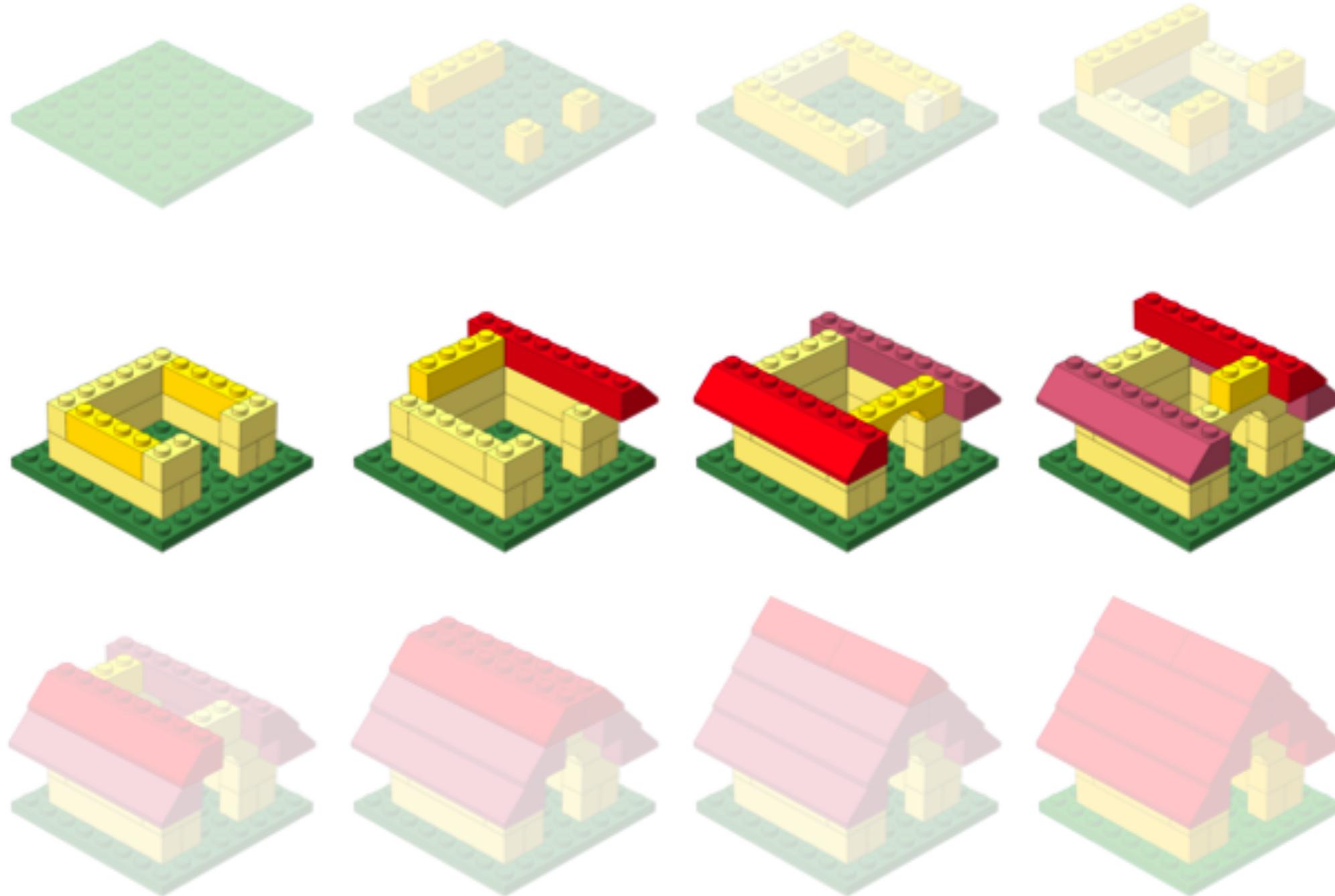
<http://anoved.net/tag/lego/page/3/>

Setting up to perform Bioinformatics analysis

Setting up...



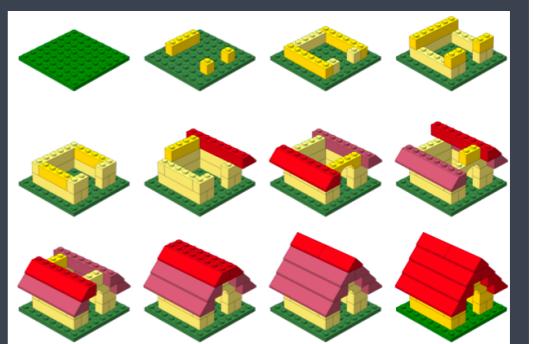
- ✓ Introduction to the command-line interface (shell, Unix, Linux)
 - Dealing with large data files
 - Performing bioinformatics analysis
 - Using tools
 - Accessing and using compute clusters
- ✓ R
 - Parsing and working with smaller results text files
 - Statistical analysis, e.g. differential expression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



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

Bioinformatics data analysis

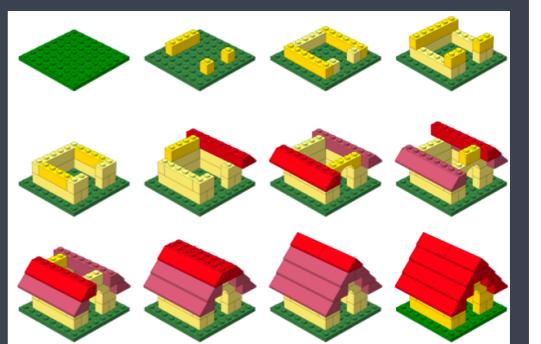
Workshop Scope



Differential Gene Expression analysis

- ✓ Understand the considerations for performing statistical analysis on RNA-seq data

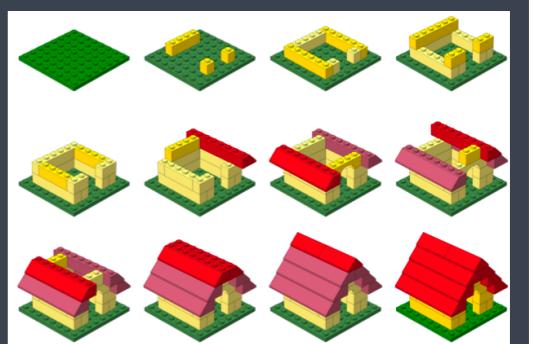
Workshop Scope



Differential Gene Expression analysis

- ✓ Understand the considerations for performing statistical analysis on RNA-seq data
- ✓ Start with gene counts (after alignment and counting)

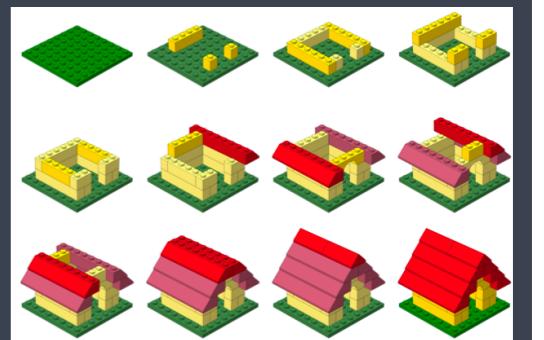
Workshop Scope



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

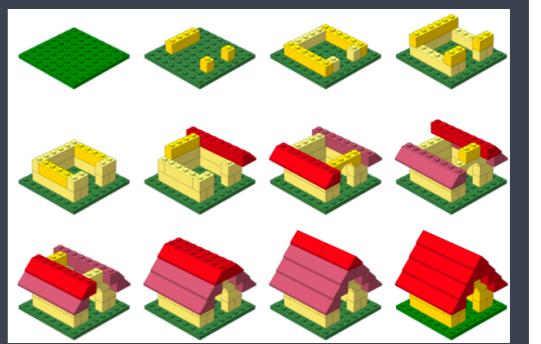
Workshop Scope



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

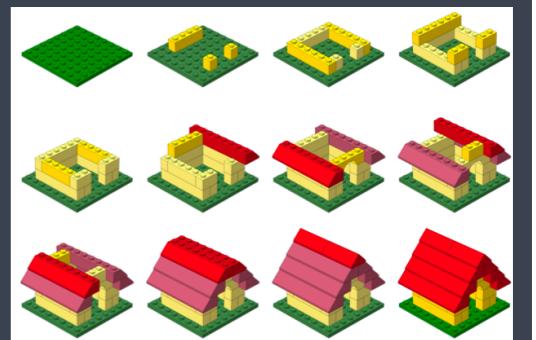
Workshop Scope



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

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	Radhika
10:30 - 10:45	R refresher Q & A	Mary
10:45 - 11:15	RNA-seq pre-reading discussion	Meeta/Mary
11:15 - 12:00	Intro to DGE / setting up DGE analysis	Meeta

Self-Learning Part 1

1. [RNA-seq counts distribution](#)
2. [Count normalization](#)
3. Sample-level QC (PCA and hierarchical clustering)

Assignment #1

- R script
- Dropbox link
- [Email us](#) about questions regarding the homework that you need answered before the next class.
- Post 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

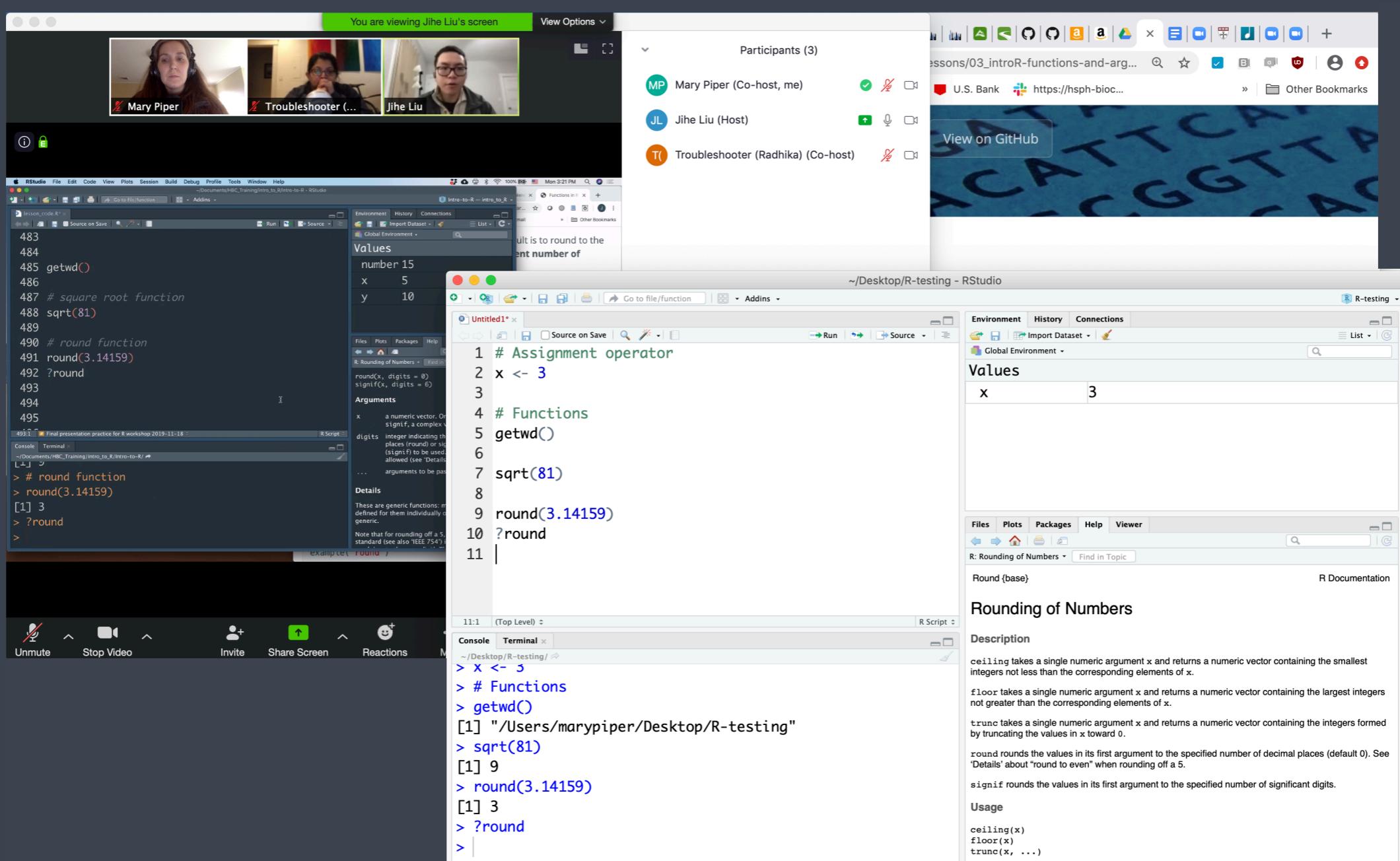
Normalization

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'.

The 3 Window problem...



The 3 Window problem...

The screenshot illustrates a video conference setup with three windows:

- Top Left Window (Zoom):** Shows three participants: Mary Piper (Co-host, me), Troubleshooter (Radhika) (Co-host), and Jihe Liu (Host). The participant list shows their names and roles.
- Bottom Left Window (RStudio):** Displays an R script in the "lesson_code.R" file. The code includes:

```
483  
484  
485 getwd()  
486  
487 # square root function  
488 sqrt(81)  
489  
490 # round function  
491 round(3.14159)  
492 ?round  
493  
494  
495
```

Console output shows:

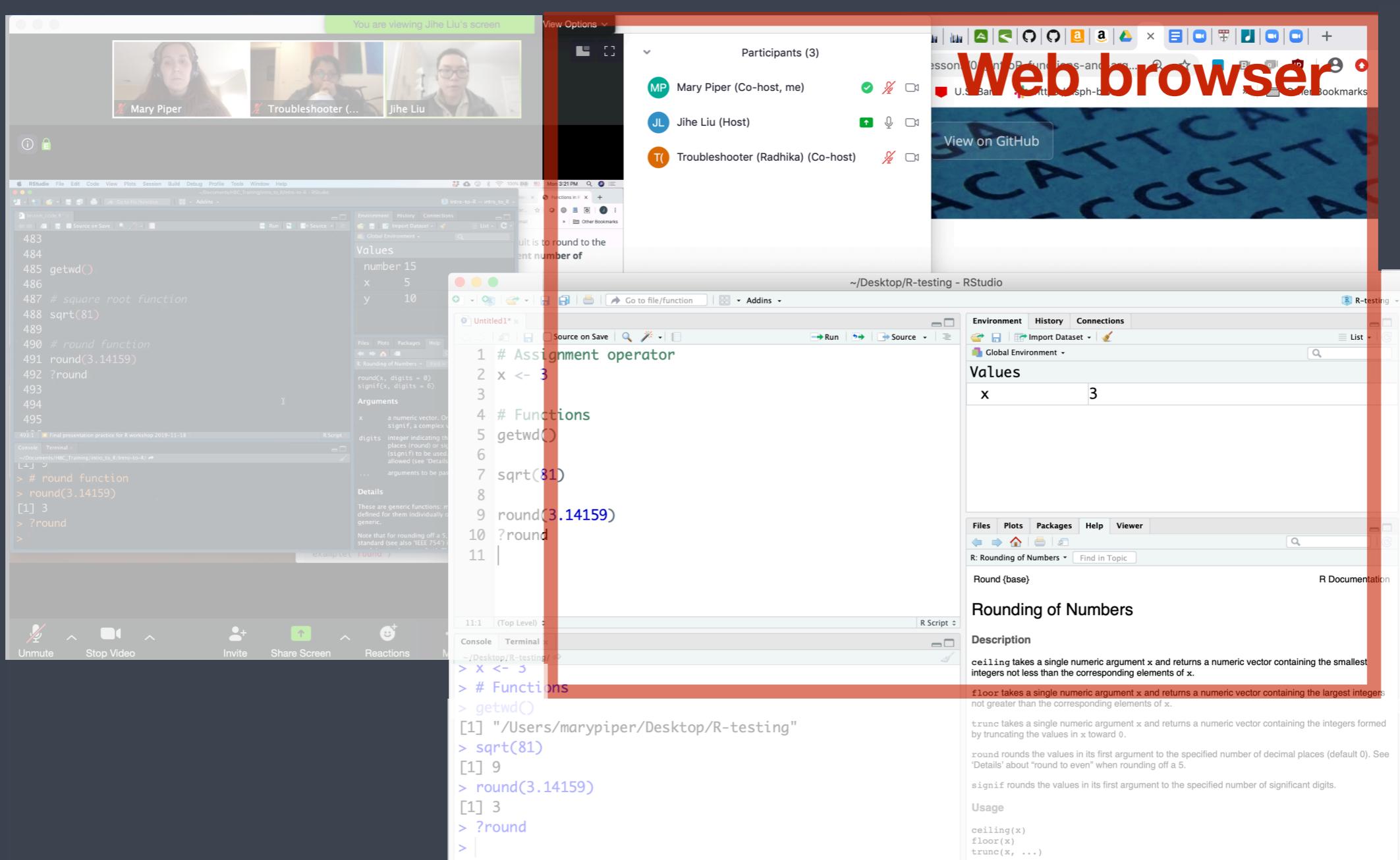
```
> # round function  
> round(3.14159)  
[1] 3  
> ?round  
>
```
- Bottom Right Window (RStudio):** Shows a single assignment in the "Untitled1" file:

```
1 # Assignment operator  
2 x <- 3  
3  
4 # Functions  
5 getwd()  
6  
7 sqrt(81)  
8  
9 round(3.14159)  
10 ?round  
11
```

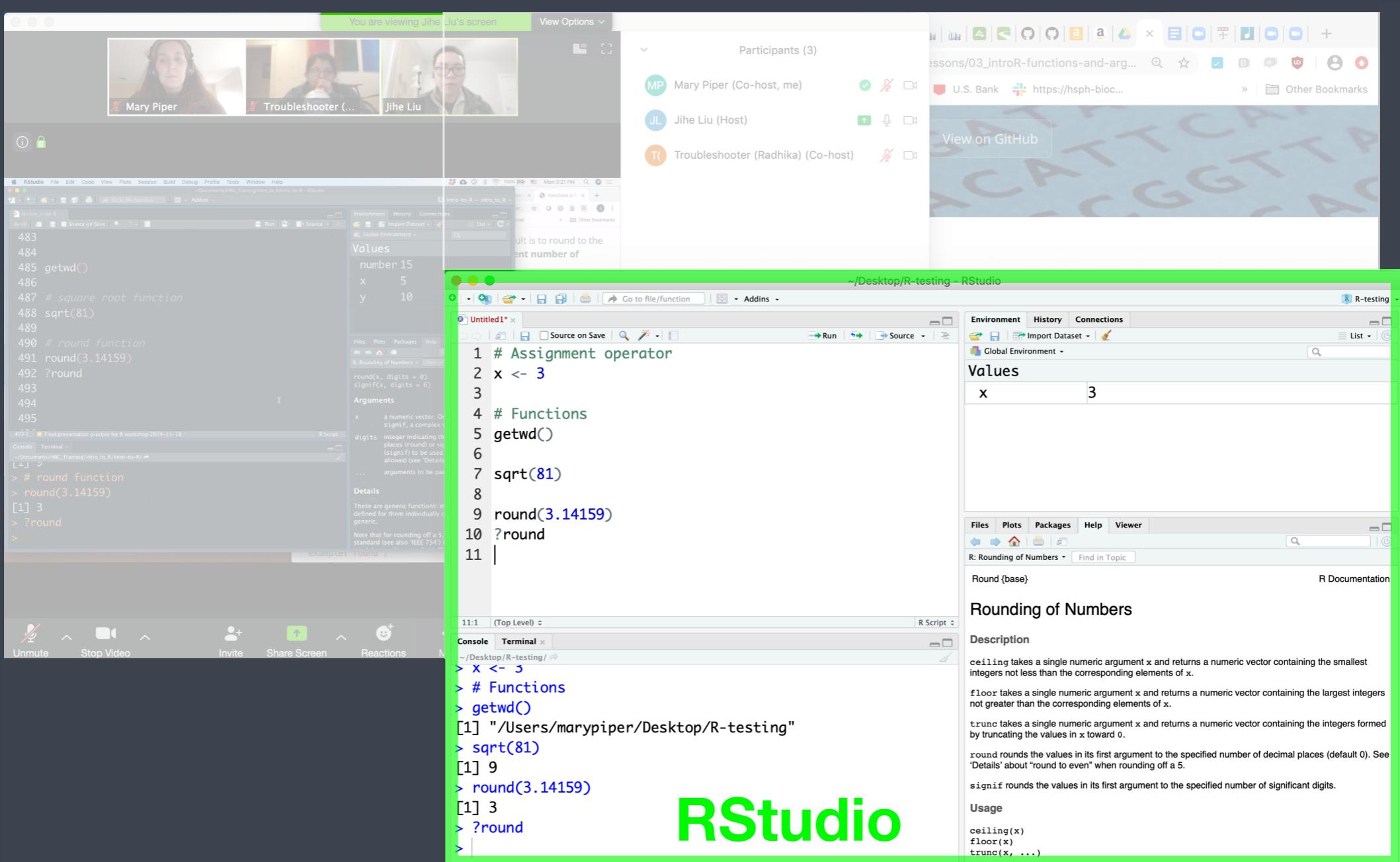
Console output shows:

```
> x <- 3  
> # Functions  
> getwd()  
[1] "/Users/marypiper/Desktop/R-testing"  
> sqrt(81)  
[1] 9  
> round(3.14159)  
[1] 3  
> ?round  
>
```
- Rightmost Window (Browser):** Displays the R Documentation page for the "R: Rounding of Numbers" package. The page includes sections for Description, Usage, and Examples.

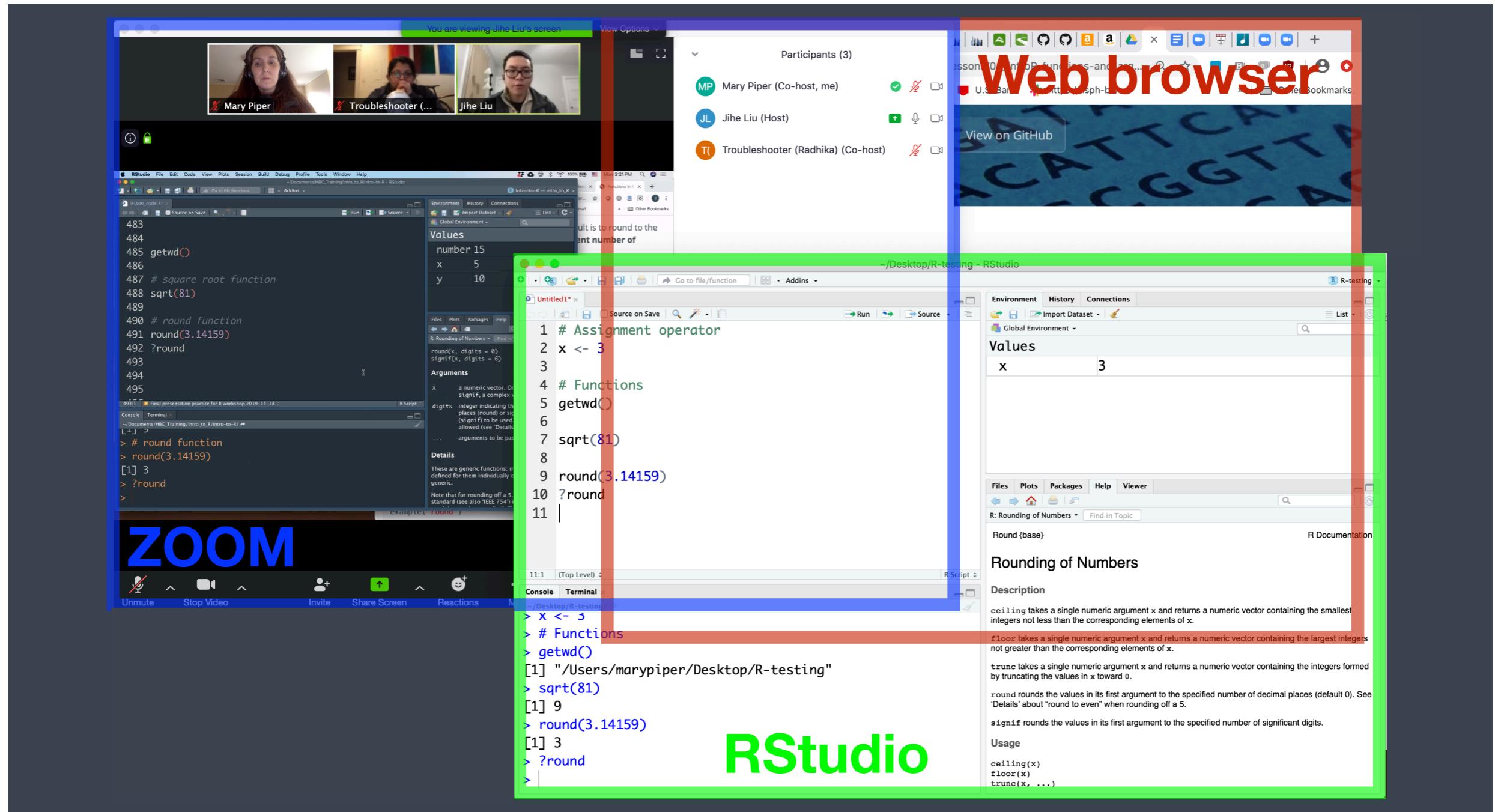
The 3 Window problem...



The 3 Window problem...

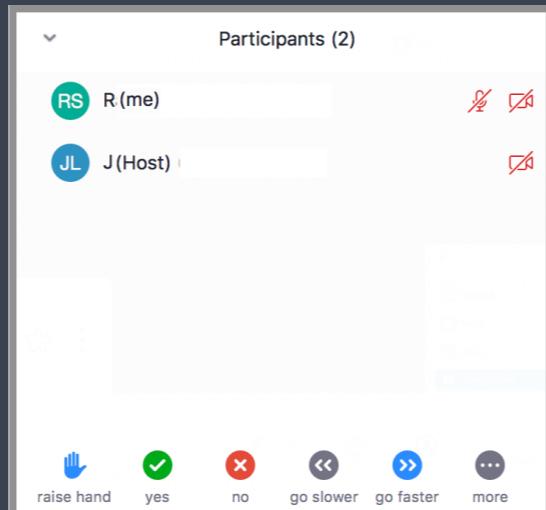


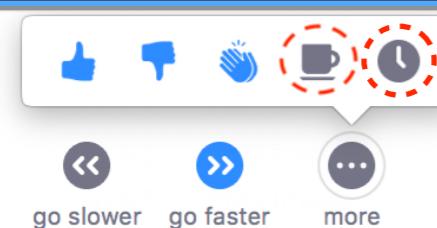
The 3 Window problem...



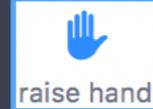
Odds and Ends (1/2)

- ❖ Quit/minimize all applications that are not required for class
- ❖ Click on “Participants” to open that panel in Zoom



- ▶  = "agree", "I'm all set" (equivalent to a **green post-it**)
- ▶  = "disagree", "I need help" (equivalent to a **red post-it**)
- ▶  If you are away from the computer use the coffee cup or clock icon

Odds and Ends (2/2)

- ❖ Questions for the presenter?
 - Post the question in the Chat window OR
 - Raise your hand  when the presenter asks for questions
- ❖ Technical difficulties with R or RStudio?
 - 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)