

# Introduction to bulk RNA-seq (Part I)

Harvard Chan Bioinformatics Core  
in collaboration with  
HMS Research Computing

<https://tinyurl.com/hbc-rnaseq>



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# Consulting

- Experimental design help
- 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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AND TRANSLATIONAL  
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# Training

A key component of the HBC's mission is to support researchers at Harvard by providing training.

HBC's training team is mainly involved in training and community building for research projects to ensure that our members have the skills and knowledge needed to succeed.

Our hands-on workshops focus on practical skills, with an emphasis on **experimental design** and **bioinformatics**, designed for **wet-lab biologists** and **bioinformaticians** who work with NGS data.

We offer three types of workshops:

1. Short, 3-hour monthly workshops
2. Basic Data Skills\*\*
3. Advanced Topics: Analysis of high-throughput sequencing (NGS) data

*\*\*The basic data skills workshops are designed for the advanced workshops.*



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Our dedicated training team holds workshops to help researchers learn how to analyze NGS data.

The training team also devote substantial time to material development, and the training team also participate in consultations on best practices in NGS analysis.

Workshops focus on the analysis of high-throughput sequencing data, with an emphasis on **experimental design**, **bioinformatics**, and **reproducibility**. Our workshops are designed for wet-lab biologists and bioinformaticians working on NGS-based experiments and analysing the resulting data.

bioinformatics)

(NGS) data\*\*

for the advanced workshops.

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

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

# Training

A key component of the HBC's mission is to support researchers at Harvard by providing training.

HBC's training team is made up of scientists who provide training and community building for research projects to ensure the quality of our work.

Our hands-on workshops focus on **bioinformatics**, with an emphasis on **experimental design** and **data analysis**. We also offer **wet-lab biologists** and **computational biologists** training in NGS data.

We offer three types of workshops:

1. Short, 3-hour monthly workshops
2. Basic Data Skills\*\*
3. Advanced Topics: Analysis of high-throughput sequencing data

*\*\*The basic data skills workshops are designed for the general public.*



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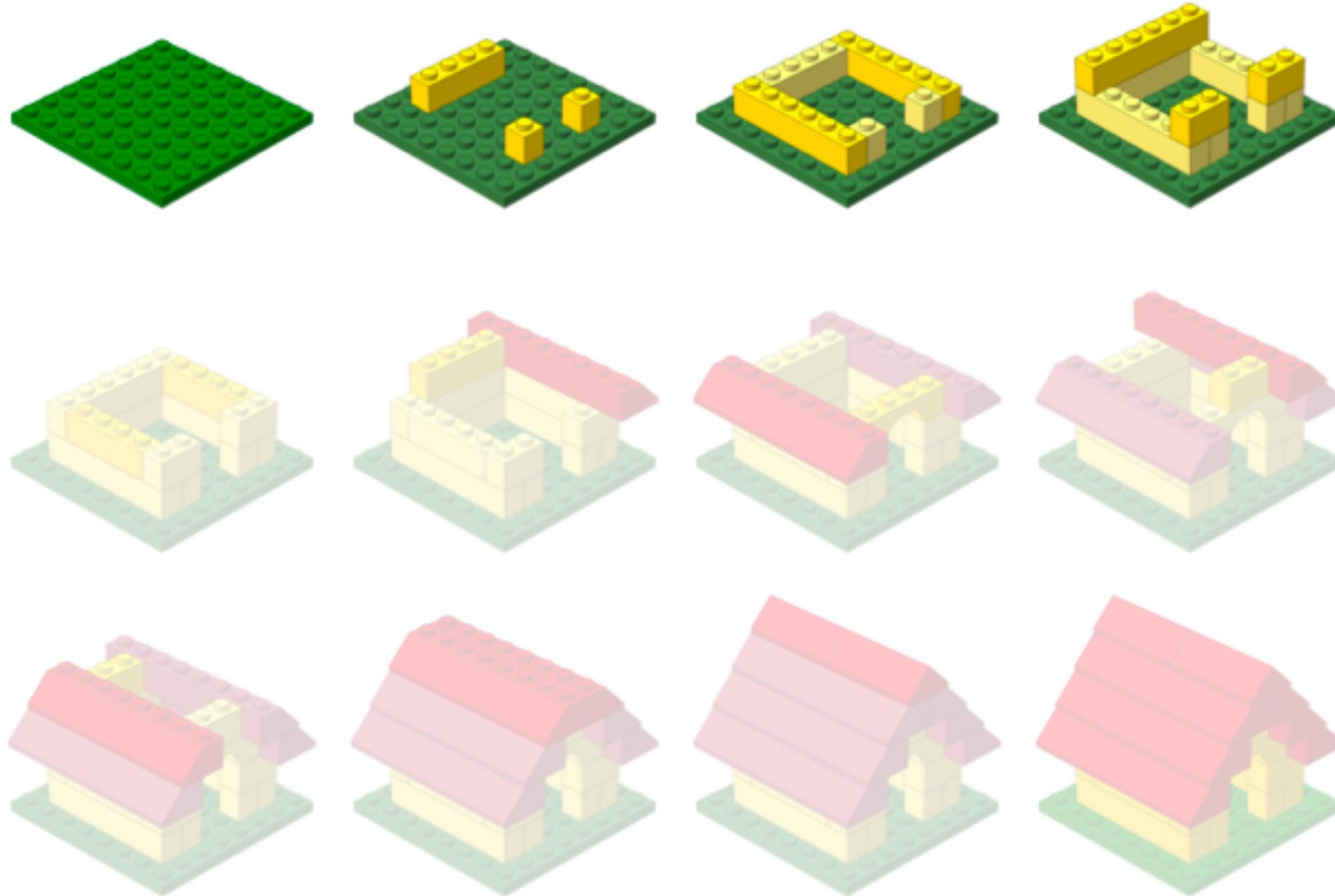
**bioinformatics**)

**NGS) data\*\***

or the advanced workshops.

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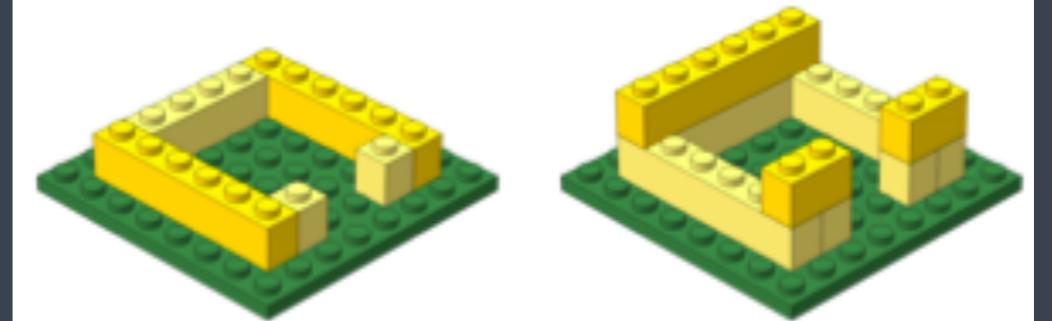
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<http://anoved.net/tag/lego/page/3/>

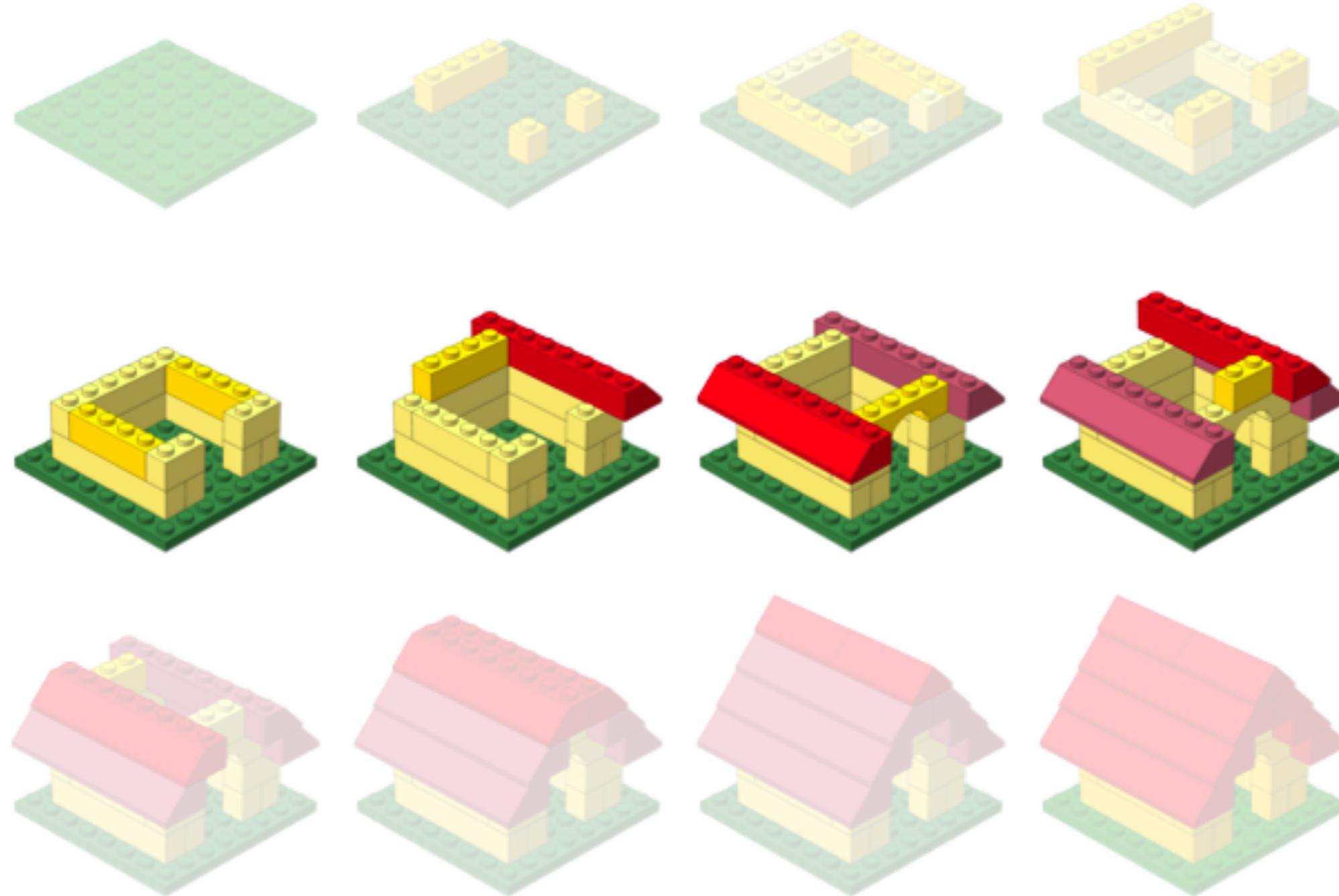
Setting up to perform Bioinformatics analysis

# 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

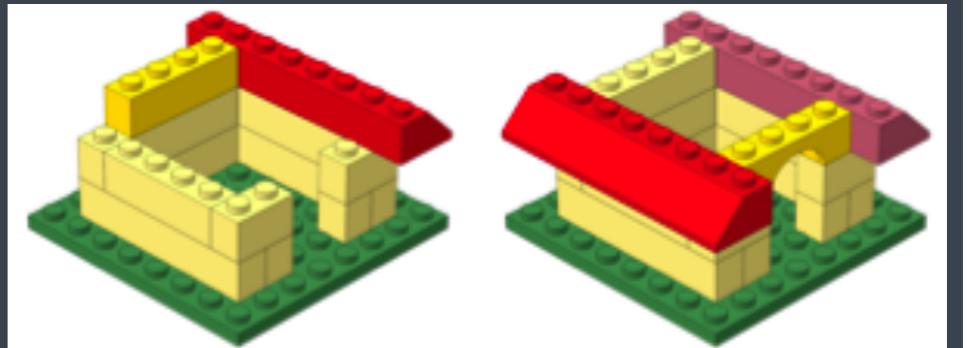
# Workshop scope



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

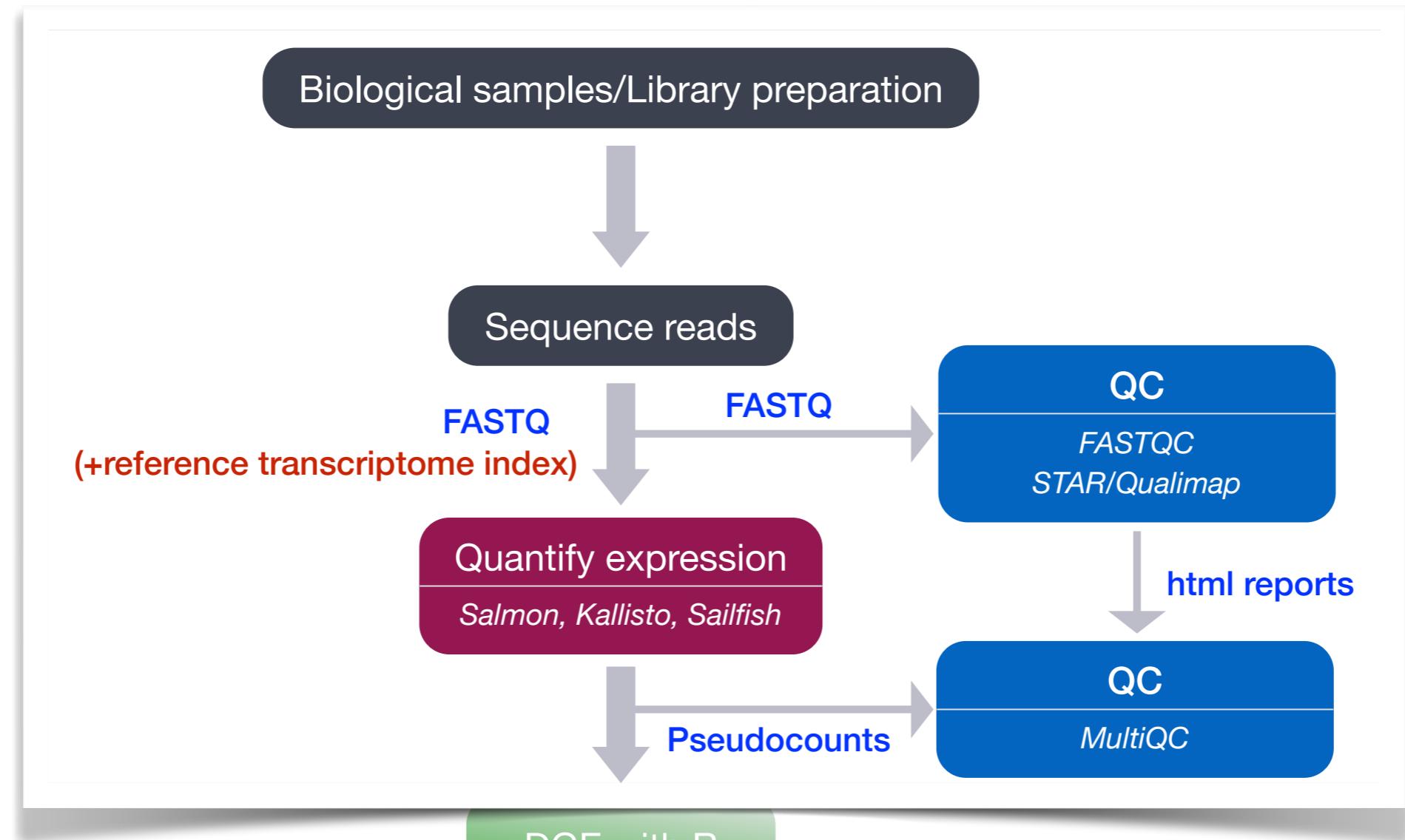
Bioinformatics data analysis

# Learning Objectives



- ✓ Describe best practices for designing a bulk RNA-seq experiment
- ✓ Describe steps in an RNA-seq analysis workflow (from sequence data to expression quantification).
- ✓ Implement shell scripts on a high-performance compute cluster to perform the above steps.

We won't be covering how to perform differential gene expression (DGE) analysis on count data in this workshop.



# The Workflow

# Logistics

# Course webpage

<https://tinyurl.com/hbc-rnaseq>

# Course schedule online

## Workshop Schedule

**NOTE:** The *Basic Data Skills Introduction to the command-line interface* workshop is a prerequisite.

### Pre-reading

- [Shell basics review](#)
- [Introduction to RNA-seq](#)

### Day 1

Time	Topic	Instructor
09:30 - 09:45	Workshop introduction	Radhika
09:45 - 10:25	Working in an HPC environment	Radhika
10:25 - 11:05	Project Organization and Best Practices in Data Management	Meeta
11:05 - 11:45	Quality Control of Sequence Data: Running FASTQC	Jihe
11:45 - 12:00	Overview of self-learning materials and homework submission	Jihe/Meeta

# Course materials online

## Introduction to RNA-Seq using high-performance computing

Intro to RNA-seq updated for a flipped classroom

[View on GitHub](#)

### Learning Objectives:

- Understand the quality values in a FASTQ file
- Create a quality report using FASTQC

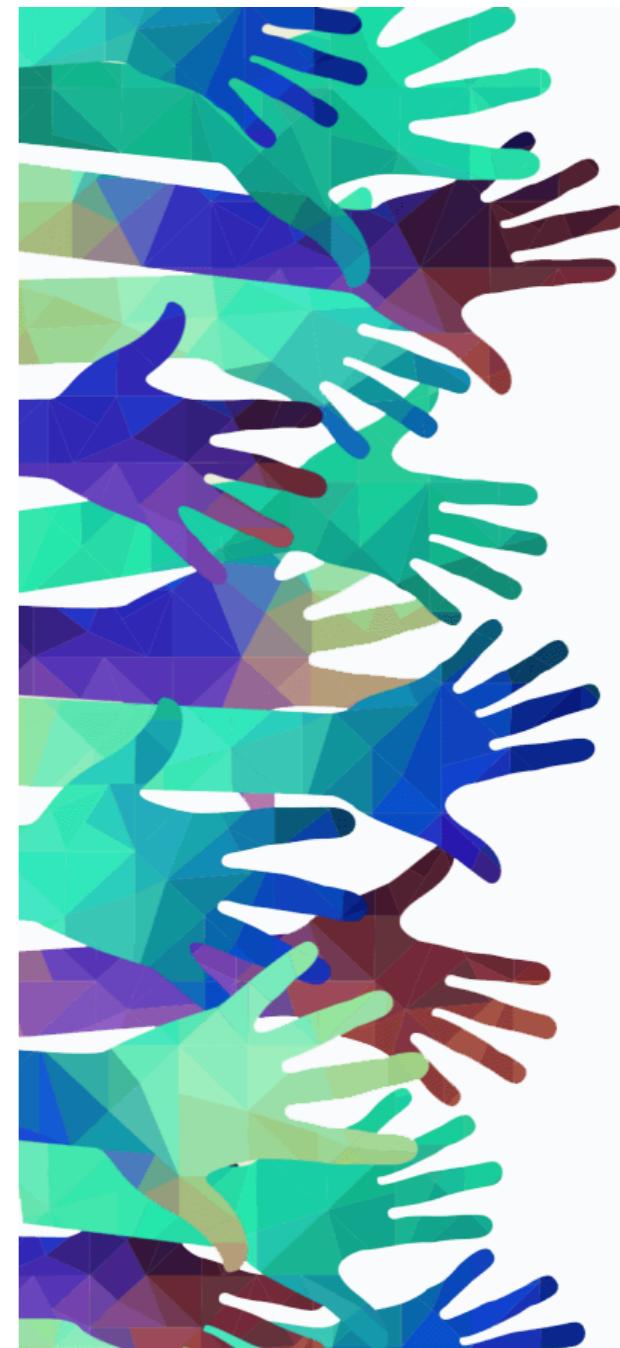
### Quality Control of FASTQ files

The first step in the RNA-Seq workflow is to take the FASTQ files received from the sequencing facility and assess the quality of the sequence reads.

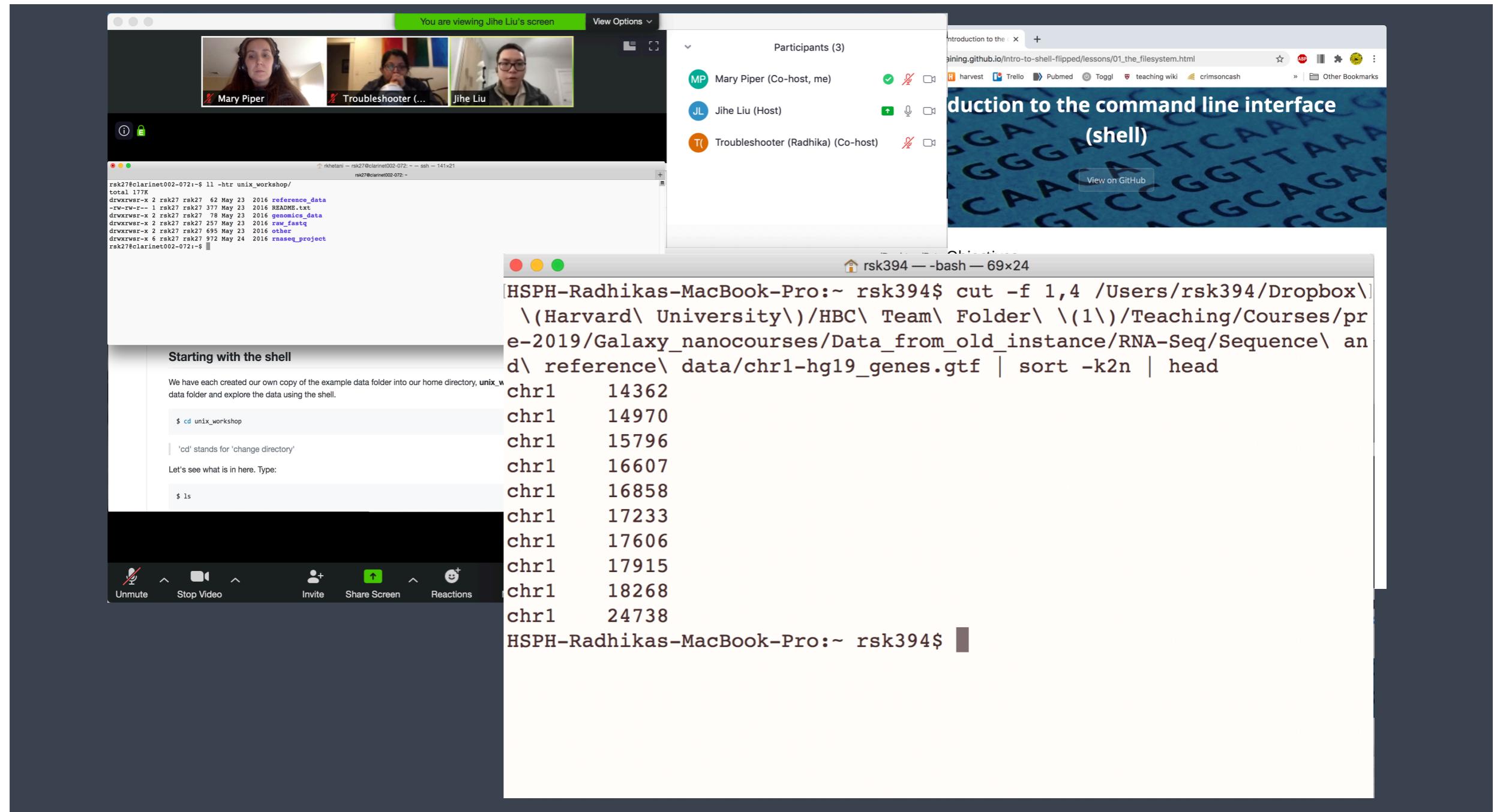
```
graph TD; A[\"Biological samples/Library preparation\"] --> B[\"Sequence reads\"]; B --> C[\"QC\"]; B --> D[\"Quantify expression\"];
```

# Course participation

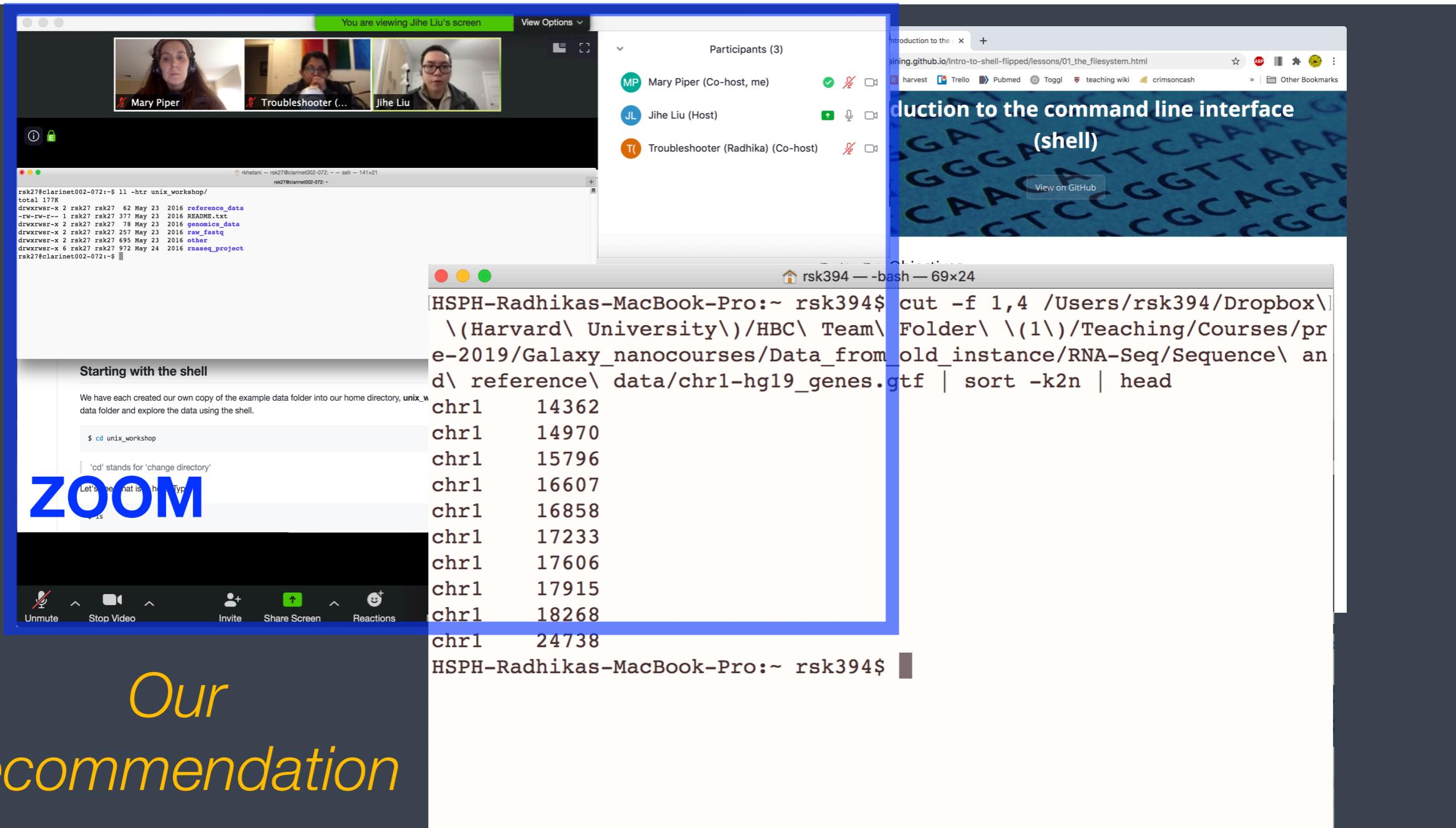
- ▶ Mandatory review of self-learning lessons and assignments
- ▶ Attendance required for all classes
- ▶ Your questions and active participation drive learning
- ▶ We look forward to all of your questions!



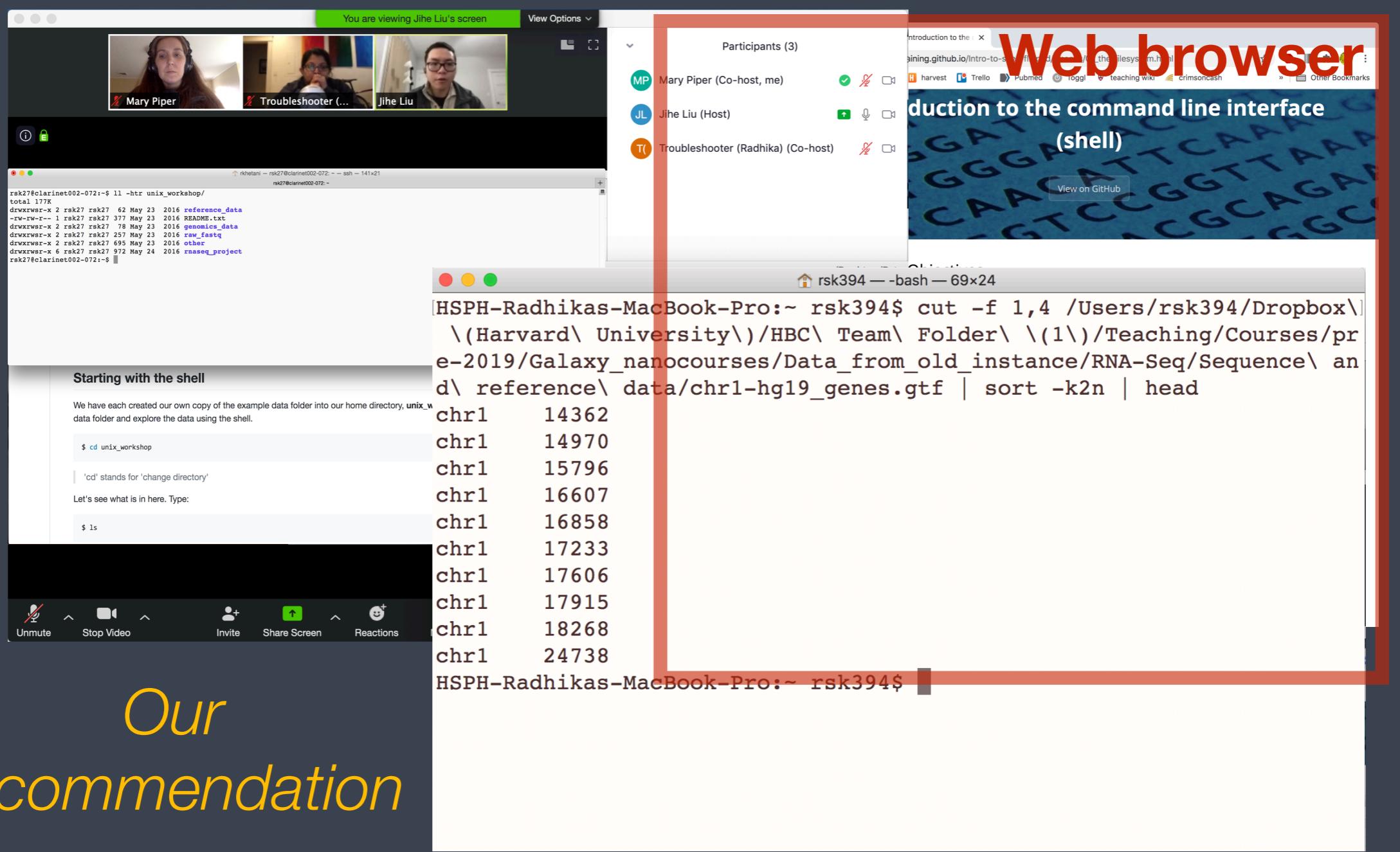
# Single screen & 3 windows?



# Single screen & 3 windows?



# Single screen & 3 windows?



# Single screen & 3 windows?

The image shows a video conference interface with three main windows:

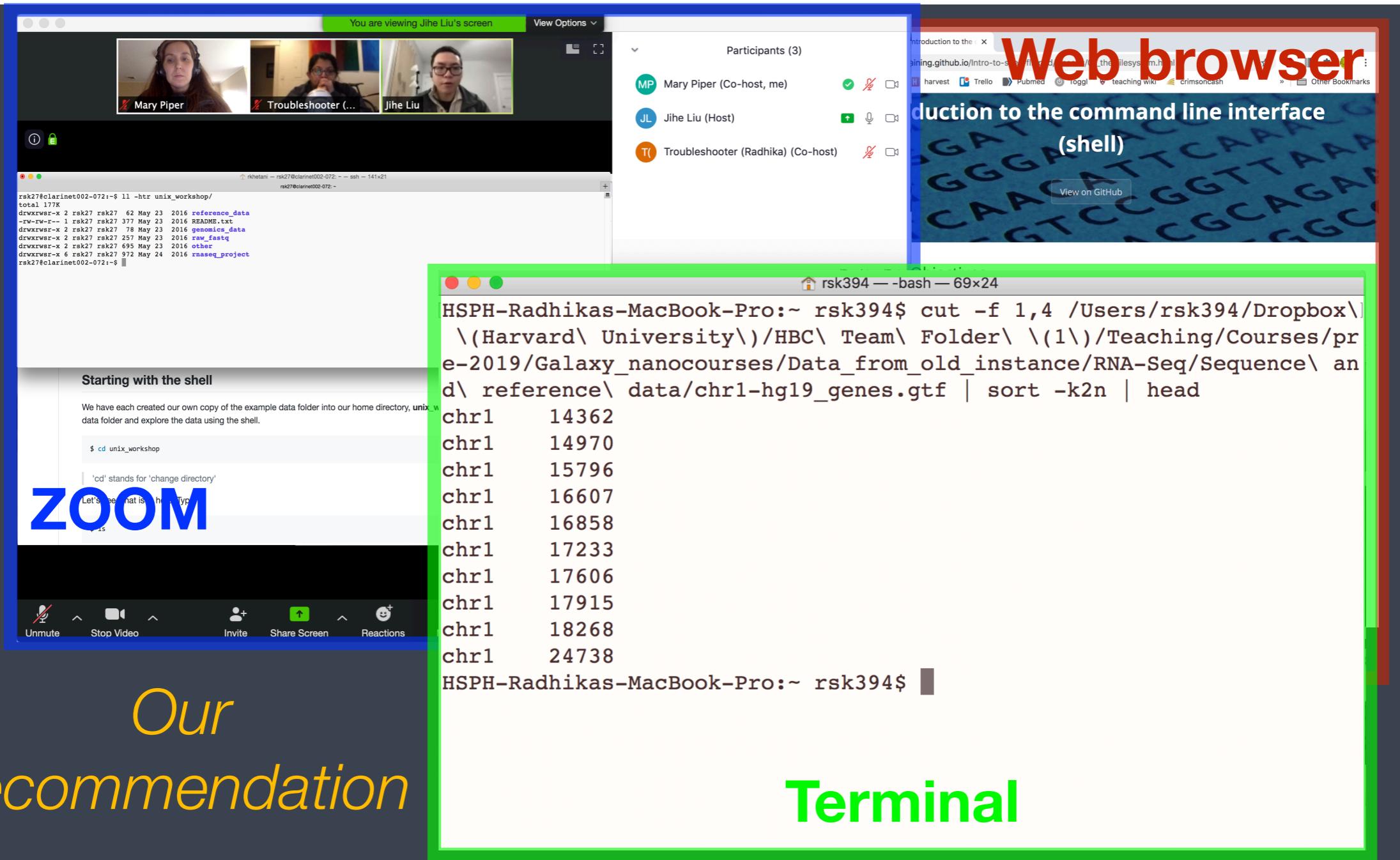
- Video Feed:** Shows three participants: Mary Piper, Troubleshooter (Radhika), and Jihe Liu.
- Participants List:** Shows three participants: Mary Piper (Co-host, me), Jihe Liu (Host), and Troubleshooter (Radhika) (Co-host).
- Terminal Session:** A green-highlighted window showing a command-line interface. The command run is:

```
rsk394 — bash — 69x24
HSPH-Radhikas-MacBook-Pro:~ rsk394$ cut -f 1,4 /Users/rsk394/Dropbox\(\Harvard\ University\)/HBC\ Team\ Folder\ \((1\))/Teaching/Courses/pre-2019/Galaxy_nanocourses/Data_from_old_instance/RNA-Seq/Sequence\ and\ reference\ data/chr1-hg19_genes.gtf | sort -k2n | head
chr1    14362
chr1    14970
chr1    15796
chr1    16607
chr1    16858
chr1    17233
chr1    17606
chr1    17915
chr1    18268
chr1    24738
```

**Our recommendation**

**Terminal**

# Single screen & 3 windows?



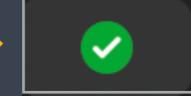
# Odds and Ends (1/2)

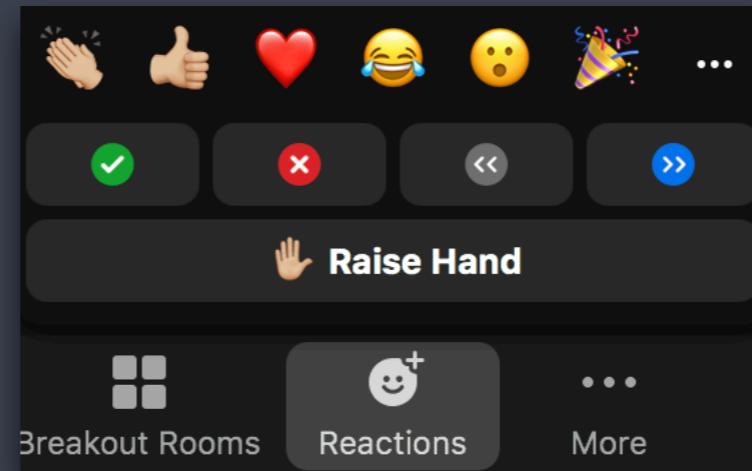
- ❖ Quit/minimize all applications that are not required for class

# Odds and Ends (1/2)

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

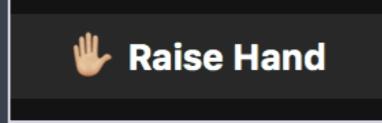
# Odds and Ends (1/2)

- ❖ 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**)



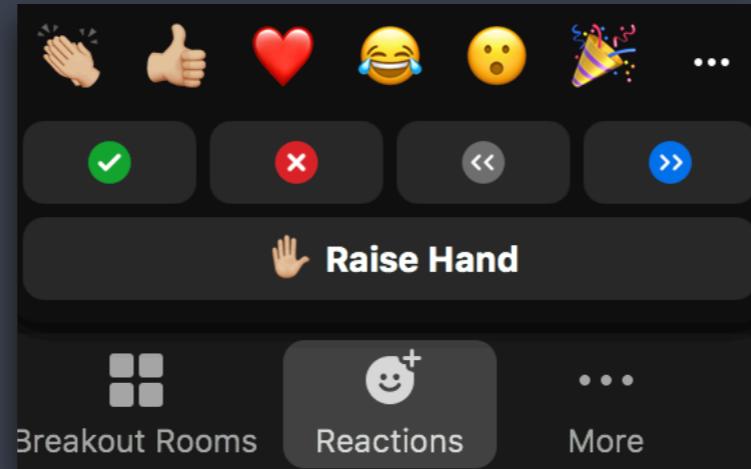
# Odds and Ends (2/2)

- ❖ Questions for the presenter?

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

- ❖ Technical difficulties with software?

- Start a private chat with the Troubleshooter with a description of the problem.



# Thanks!

- Andy Bergman & Kathleen Chappell from HMS-RC
- [Data Carpentry](#)

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# Contact us!

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