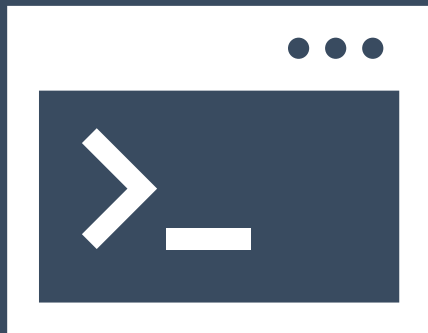


Intro to Bulk RNA-seq (Part I)

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



Harvard Chan Bioinformatics Core



Introductions!





Shannan Ho Sui
Director



Meeta Mistry
Associate Director



Lorena Pantano
*Director of Bioinformatics
Platform*



John Quackenbush
Faculty Advisor



Upen Bhattarai



Heather Wick



Will Gammerdinger



Noor Sohail



Alex Bartlett



Elizabeth
Partan



Emma Berdan



James Billingsley



Zhu Zhuo



Maria Simoneau



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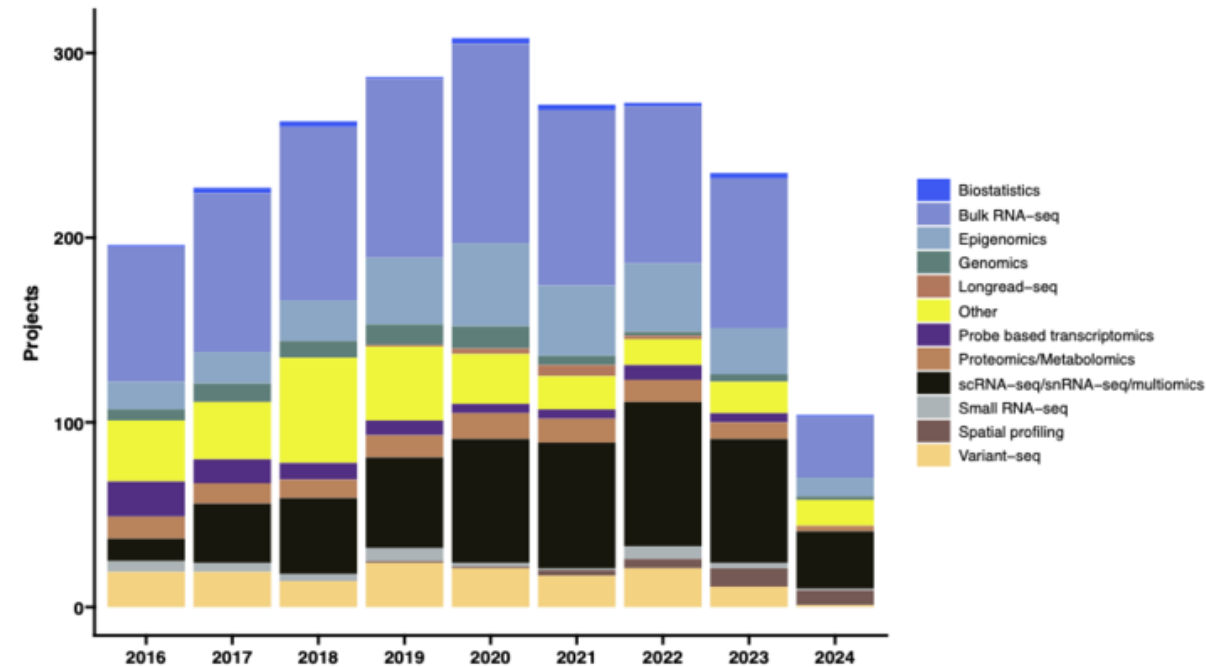
Zhu Zhuo



Maria Simoneau

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



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HARVARD
T.H. CHAN
SCHOOL OF PUBLIC HEALTH

NIEHS



THE HARVARD CLINICAL
AND TRANSLATIONAL
SCIENCE CENTER



HARVARD
MEDICAL SCHOOL

Training

A key component of the HBC's mission is its training initiative. Our dedicated training team holds workshop to help researchers at Harvard better understand analytical methods for NGS data.

[HBC's training team](#) is made up of four PhD-level scientists who devote substantial time to material development, training and community building/outreach. All members of the training team also participate in consultations on research projects to ensure they remain up-to-date on current best practices in NGS analysis.

Our hands-on workshops focus on **basic data skills** and **analysis of high-throughput sequencing data**, with an emphasis on **experimental design**, current **best practices** and **reproducibility**. Our workshops are designed for **wet-lab biologists** aiming to independently design sequencing-based experiments and analysing the resulting data.

We offer three types of workshops:

1. [Short, 3-hour monthly workshops](#) (*Current topics in bioinformatics*)
2. [Basic Data Skills](#)**
3. [Advanced Topics: Analysis of high-throughput sequencing \(NGS\) data](#)**

***The basic data skills workshops serve as the foundation for the advanced workshops.*

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

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SCHOOL OF PUBLIC HEALTH

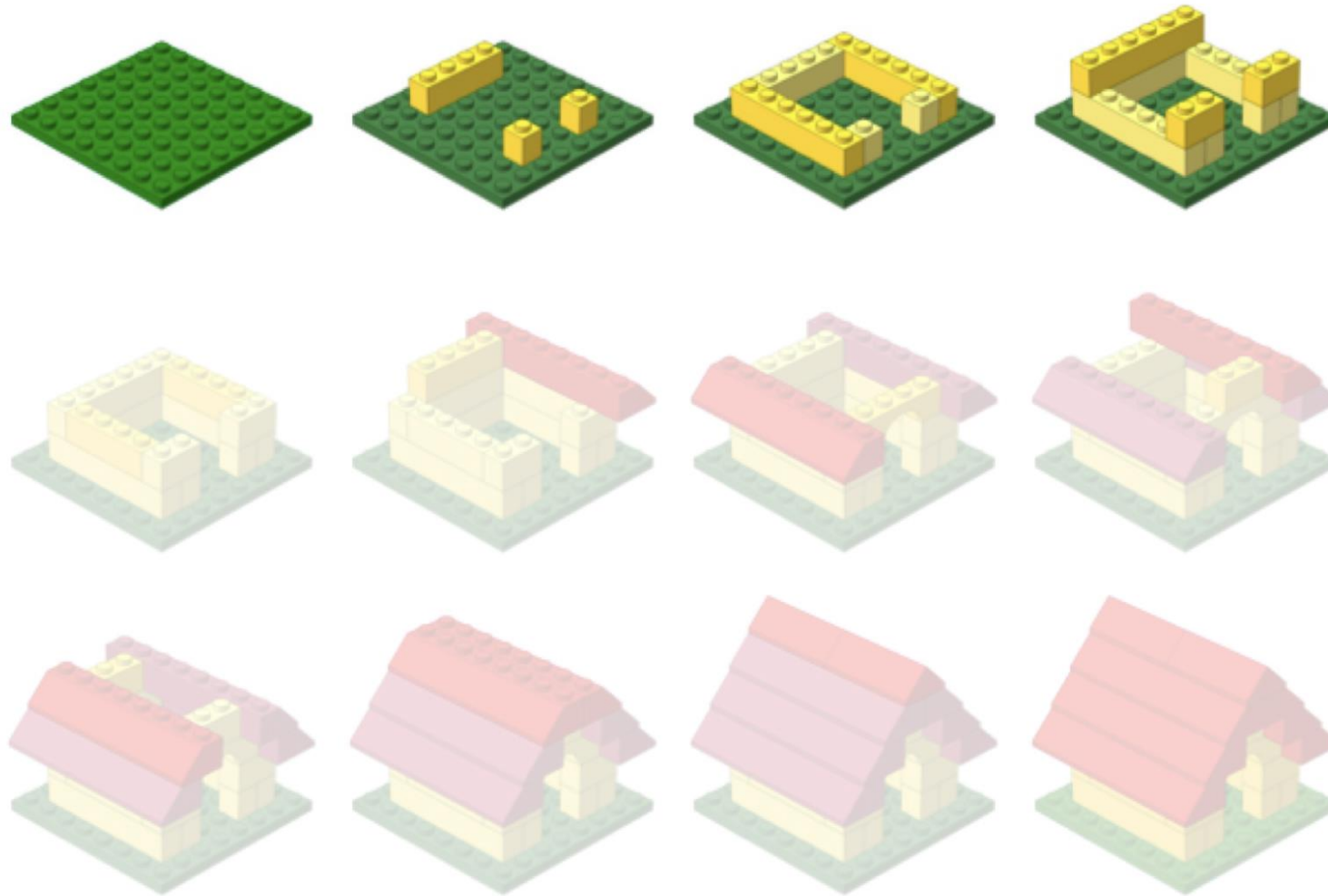
DF/HCC
DANA-FARBER / HARVARD CANCER CENTER



THE HARVARD CLINICAL
AND TRANSLATIONAL
SCIENCE CENTER

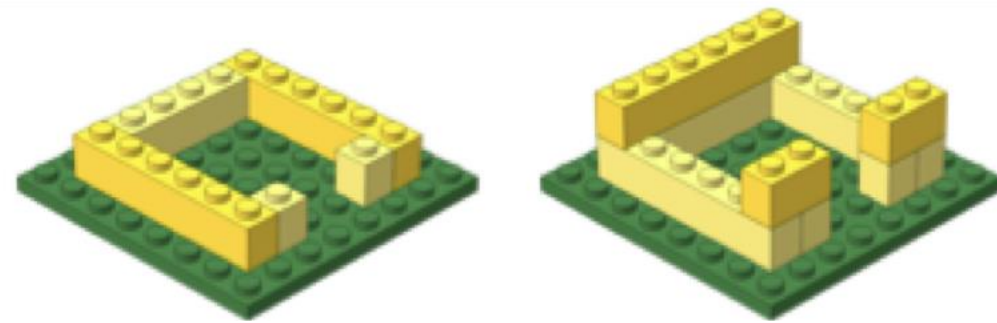


HARVARD
MEDICAL SCHOOL



Learning Bioinformatics

Setting up



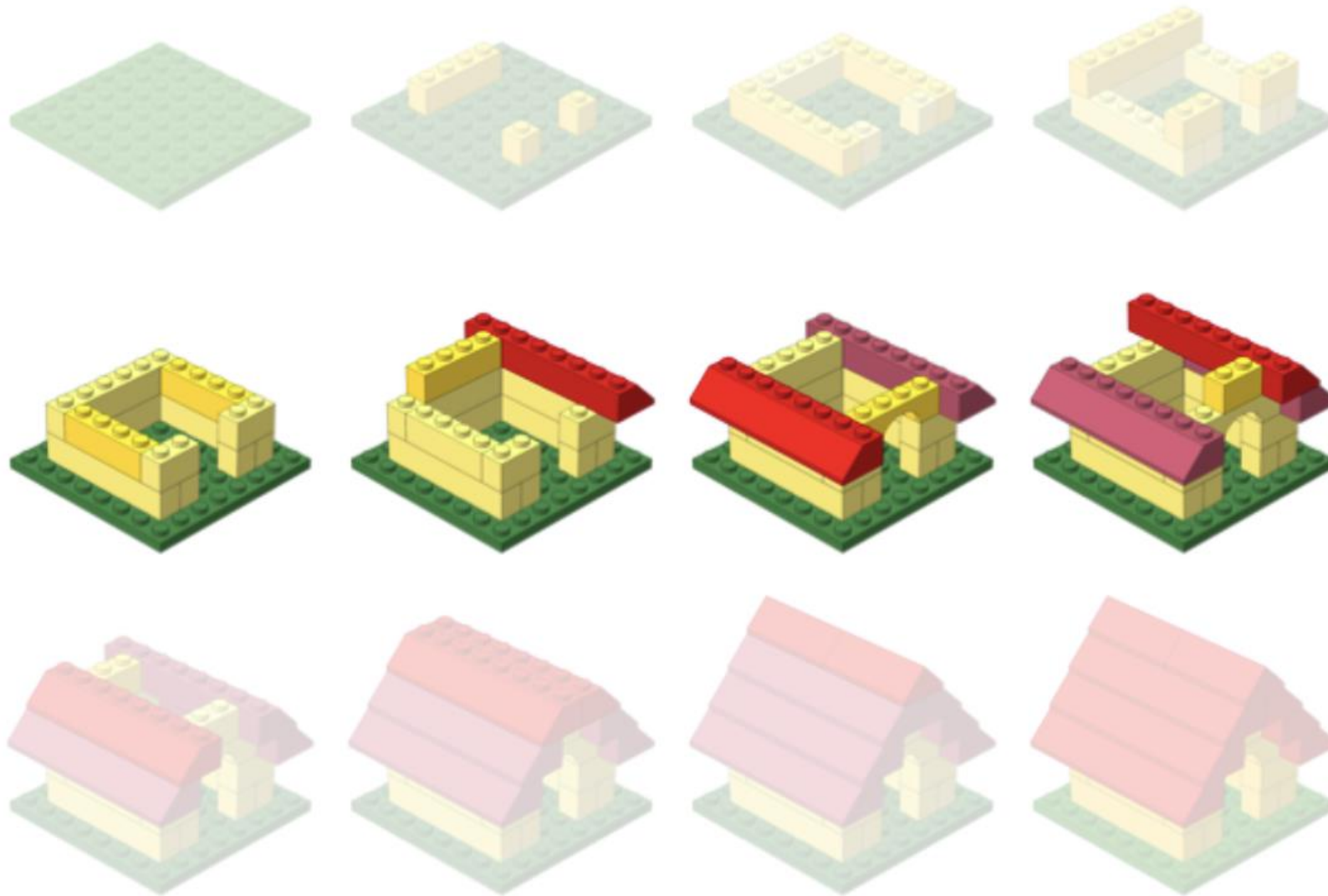
❖ Shell for Bioinformatics

- ❖ Dealing with large data files
- ❖ Performing Bioinformatic Analyses
 - ❖ Using tools
 - ❖ Accessing and using computer clusters

❖ Introduction to R

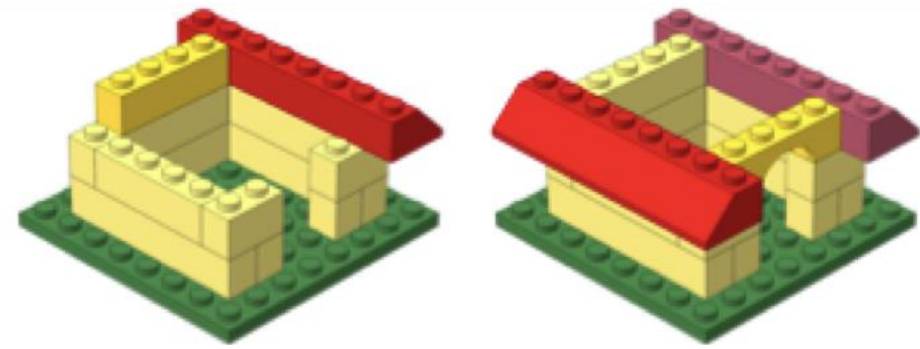
- ❖ Parsing and working with smaller results text files
- ❖ Statistical analyses, e.g. differential expression analysis
- ❖ Generating figures from complex data

Workshop scope



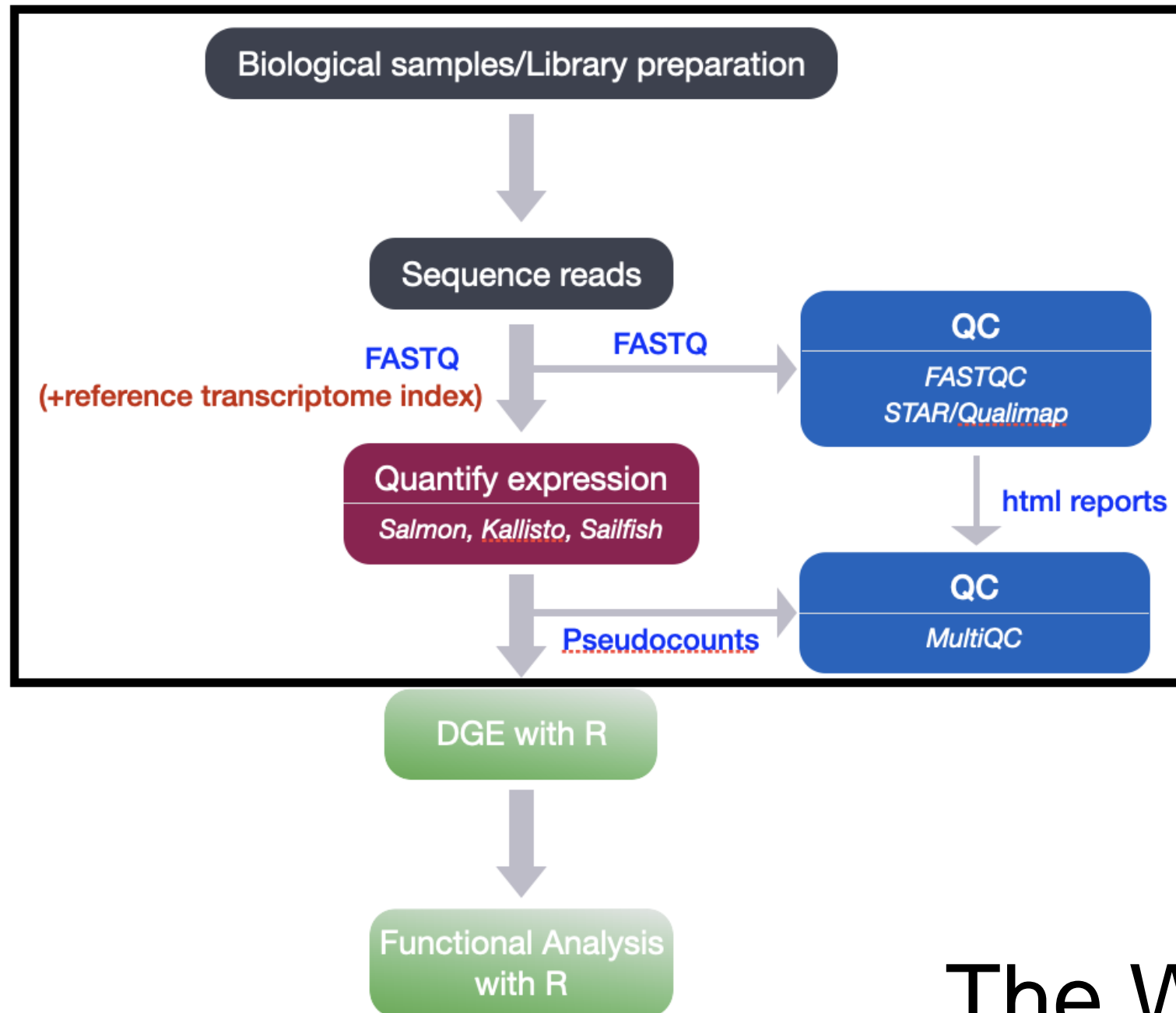
Bioinformatics Data Analysis

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

Day 1

Time	Topic	Instructor
09:30 - 09:45	Workshop Introduction	Will
09:45 - 10:25	Working in an HPC environment - Review	Upen
10:25 - 11:05	Project Organization (using Data Management best practices)	Will
11:05 - 11:45	Quality Control of Sequence Data: Running FASTQC	Upen
11:45 - 12:00	Overview of self-learning materials and homework submission	Will

Before the next class:

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

- Experimental design considerations
- Quality Control of Sequence Data: Running FASTQC on multiple samples
- Quality Control of Sequence Data: Evaluating FASTQC reports

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

Course materials

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



Learning Objectives

- Describe the example RNA-seq experiment and its objectives.
- Demonstrate strategies for good data management and project organization.

The Dataset

The dataset we are using for this workshop is part of a larger study described in [Kenny PJ et al., *Cell Rep* 2014](#). The authors are investigating interactions between various genes involved in Fragile X syndrome, a disease of aberrant protein production, which results in cognitive impairment and autistic-like features. **The authors sought to show that RNA helicase MOV10 regulates the translation of RNAs involved**

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

Single Screen & 3 Windows

The image illustrates a single-screen setup for a video call and a terminal window. The Zoom interface shows three participants: Mary Piper (Co-host, me), Jihe Liu (Host), and Troubleshooter (Radhika) (Co-host). The terminal window, titled 'rsk394', displays the following command and output:

```
HSPH-Radhikas-MacBook-Pro:~ rsk394$ cut -f 1,4 /Users/rsk394/Dropbox\
\ (Harvard\ University\)/HBC\ Team\ Folder\ \ (1\)/Teaching/Courses/pr
e-2019/Galaxy_nanocourses/Data_from_old_instance/RNA-Seq/Sequence\ an
d\ reference\ data/chr1-hg19_genes.gtf | sort -k2n | head
```

The output of the command is a list of chromosome and gene coordinates:

```
chr1 14362
chr1 14970
chr1 15796
chr1 16607
chr1 16858
chr1 17233
chr1 17606
chr1 17915
chr1 18268
chr1 24738
```

The terminal window also shows the prompt 'HSPH-Radhikas-MacBook-Pro:~ rsk394\$'.

Single Screen & 3 Windows

Zoom

Starting with the shell

We have each created our own copy of the example data folder into our home directory, `unix_w` data folder and explore the data using the shell.

```
$ cd unix_workshop
```

'cd' stands for 'change directory'

Let's see what is in here. Type:

```
$ ls
```

Participants (3)

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

Introduction to the command line interface (shell)

View on GitHub

```
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.chr1 | sort -k2n | head
```

```
chr1 14362
chr1 14970
chr1 15796
chr1 16607
chr1 16858
chr1 17233
chr1 17606
chr1 17915
chr1 18268
chr1 24738
```

HSPH-Radhikas-MacBook-Pro:~ rsk394\$

Our Recommendation

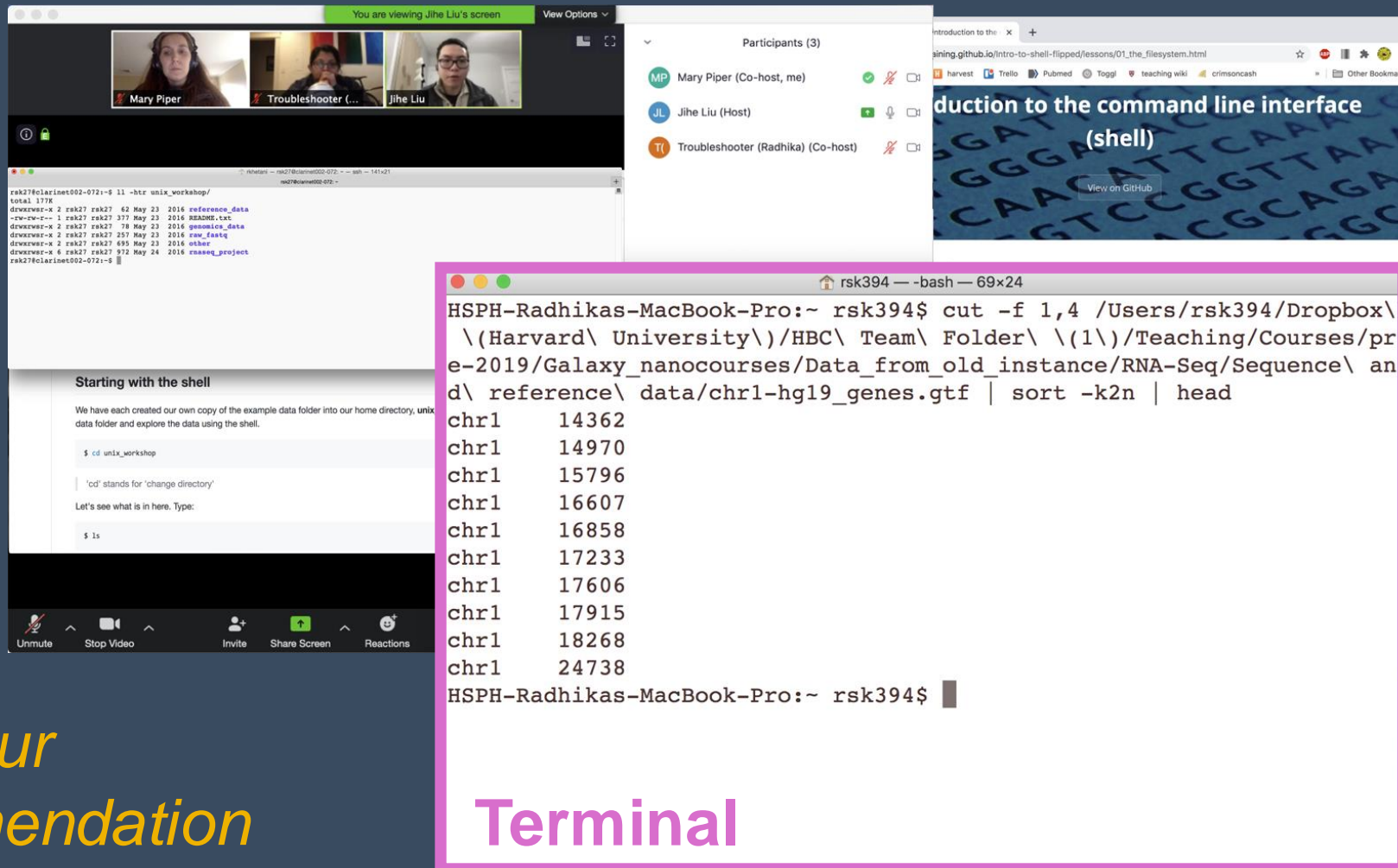
Single Screen & 3 Windows

The screenshot illustrates a Zoom meeting interface with a single screen displaying three windows. The top window shows a video feed of three participants: Mary Piper, Troubleshooter (...), and Jihe Liu. The middle window displays a list of participants: Mary Piper (Co-host, me), Jihe Liu (Host), and Troubleshooter (Radhika) (Co-host). The bottom window shows a web browser displaying a command line interface (shell) with the text "Introduction to the command line interface (shell)" and "Web Browser". The command line interface shows a terminal window with the following commands and output:

```
rsk394@HSPH-Radhikas-MacBook-Pro:~$ cut -f 1,4 /Users/rsk394/Dropbox/
\ (Harvard\ University\)/HBC\ Team\ Folder\ \ (1\)/Teaching/Courses/pr
e-2019/Galaxy_na
ocourses/Data_from_old_instance/RNA-Seq/Sequence\ an
a\ 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
HSPH-Radhikas-MacBook-Pro:~$ rsk394$
```

Below the screenshot, the text "Our Recommendation" is written in a yellow, stylized font.

Single Screen & 3 Windows



The image illustrates a single-screen setup for a video conference and terminal interaction. The top section shows a video conference interface with three participants: Mary Piper, Troubleshooter (Radhika), and Jihe Liu. Below the video feed, a terminal window is open, displaying a command and its output. The command is:

```
HSPH-Radhikas-MacBook-Pro:~ rsk394$ cut -f 1,4 /Users/rsk394/Dropbox\ \ (Harvard\ University\)/HBC\ Team\ Folder\ \ (1\)/Teaching/Courses/pr e-2019/Galaxy_nanocourses/Data_from_old_instance/RNA-Seq/Sequence\ and\ reference\ data/chr1-hg19_genes.gtf | sort -k2n | head
```

The output of the command is:

```
chr1 14362
chr1 14970
chr1 15796
chr1 16607
chr1 16858
chr1 17233
chr1 17606
chr1 17915
chr1 18268
chr1 24738
```

The terminal window is titled 'rsk394' and shows the command prompt 'HSPH-Radhikas-MacBook-Pro:~ rsk394\$'. The video conference interface also shows a 'Participants' list with three participants: Mary Piper (Co-host, me), Jihe Liu (Host), and Troubleshooter (Radhika) (Co-host). The interface includes controls for Unmute, Stop Video, Invite, Share Screen, and Reactions.

The bottom section of the image features the text 'Our Recommendation' in yellow and 'Terminal' in purple.

Single Screen & 3 Windows

The image is a composite of three overlapping windows from a Zoom meeting. The background window is the Zoom interface, showing a video feed of three participants (Mary Piper, Troubleshooter (...), and Jihe Liu) and a list of participants on the right. The title bar says "You are viewing Jihe Liu's screen". The foreground window is a terminal window titled "HSPH-Radhikas-MacBook-Pro:~ rsk394\$". It shows a command prompt and a list of file paths. The third window is a web browser window titled "Introduction to the command line interface (shell)". It shows a GitHub page with the title "Introduction to the command line interface (shell)" and the text "Web Browser".

Zoom

Web Browser

Terminal

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

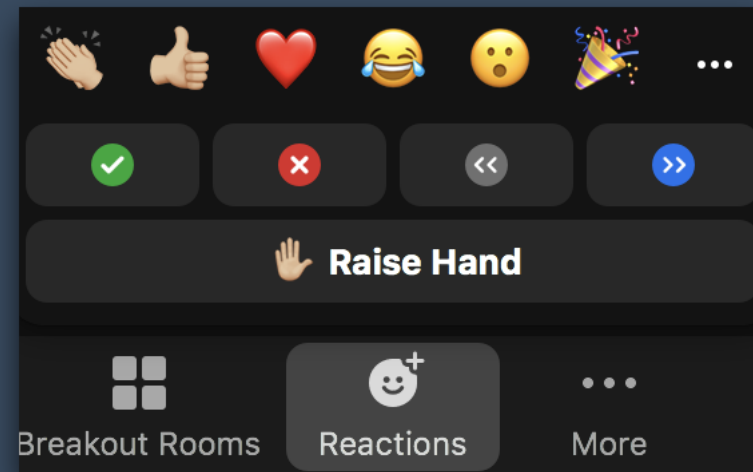
Odds & Ends

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

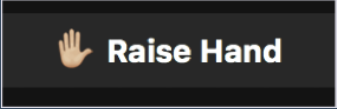
❖ Are you all set?

❖  = "agree", "I'm all set"

❖  = "disagree", "I need help"



Odds & Ends

- ❖ Questions for the presenter?
 - ❖ Post the question in the Chat window OR
 - ❖  when the presenter asks for questions
 - ❖ Let the Troubleshooter know

Odds & Ends

❖ Questions for the presenter?

- ❖ Post the question in the Chat window OR

- ❖  when the presenter asks for questions

- ❖ Let the Troubleshooter know

❖ Technical difficulties with software?

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

Thanks!

- ❖ Kathleen Chappell and Andy Bergman from HMS-RC
- ❖ Data Carpentry

These materials have been developed by members of the teaching team at the Harvard Chan Bioinformatics Core (HBC). These are open access materials distributed under the terms of the Creative Commons Attribution license (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Contact Us

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- ❖ *O2 (HMS-RC):* rchelp@hms.harvard.edu