HBC
Harvard Chan Bioinformatics Core

# Understanding chromatin biology using high throughput sequencing methods

https://tinyurl.com/hbc-chipseq

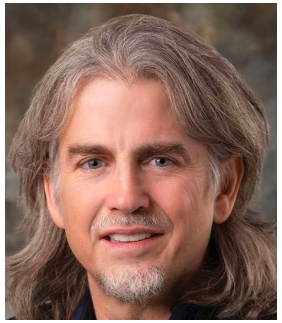
Harvard Chan Bioinformatics Core
in collaboration with
HMS Research Computing

# Introductions!

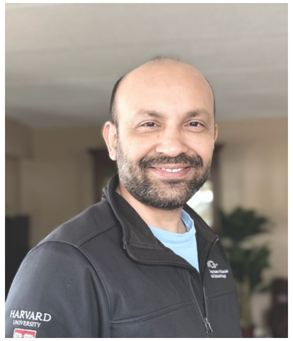
Shannan Ho Sui
*Director*

Meeta Mistry
*Associate Director*

Lorena Pantano
*Director of Bioinformatics Platform*



John Quackenbush
*Faculty Advisor*



Upen Bhattarai

Heather Wick

Will Gammerdinger

Noor Sohail

Elizabeth Partan

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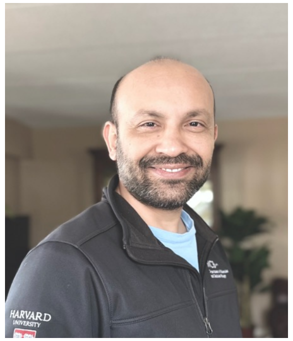

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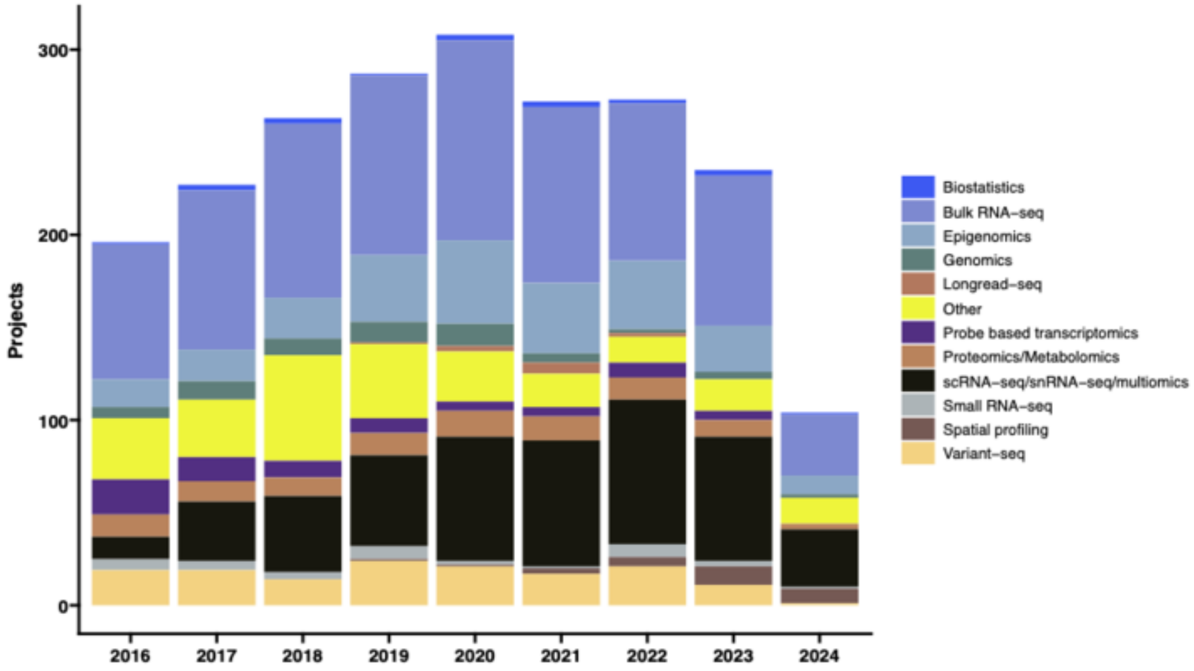
# Consulting

- 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

VE RI TAS
HARVARD
T.H. CHAN
SCHOOL OF PUBLIC HEALTH

NIEHS

HARVARD
CATALYST
THE HARVARD CLINICAL AND TRANSLATIONAL SCIENCE CENTER

VE RI TAS
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

**https://bioinformatics.sph.harvard.edu/training**

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DF/HCC
DANA-FARBER / HARVARD CANCER CENTER

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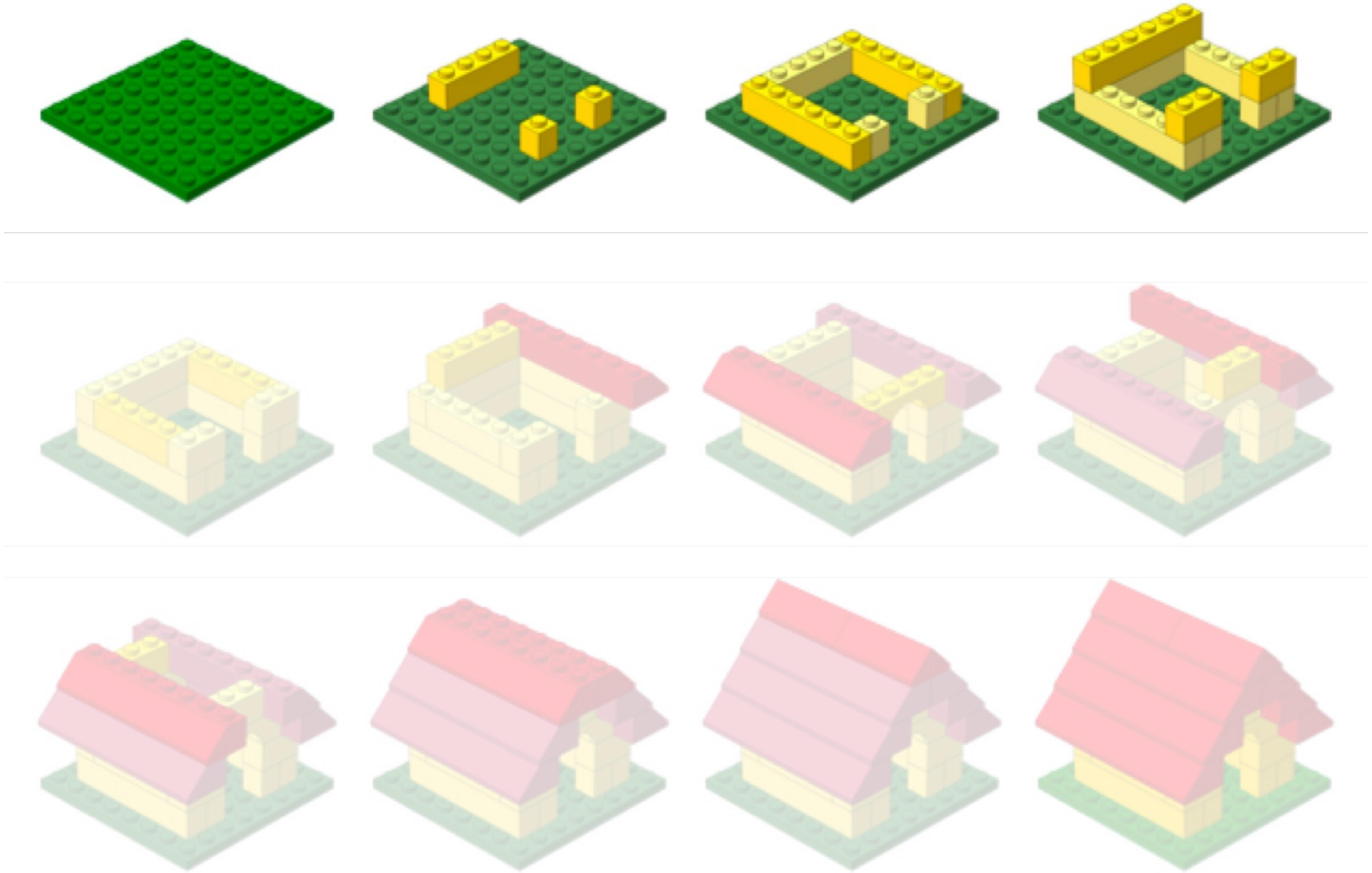
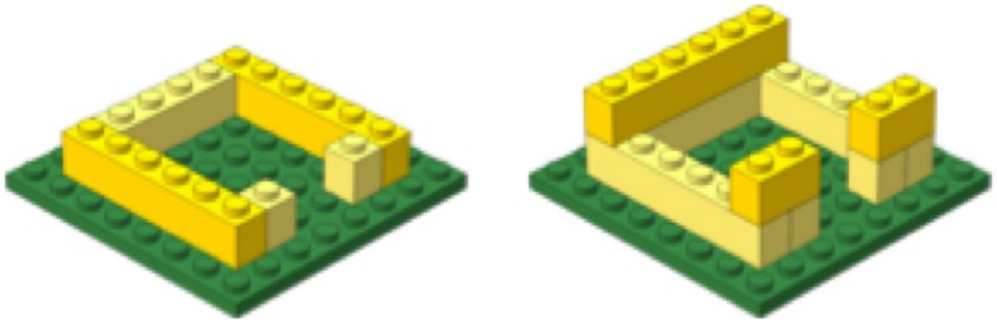


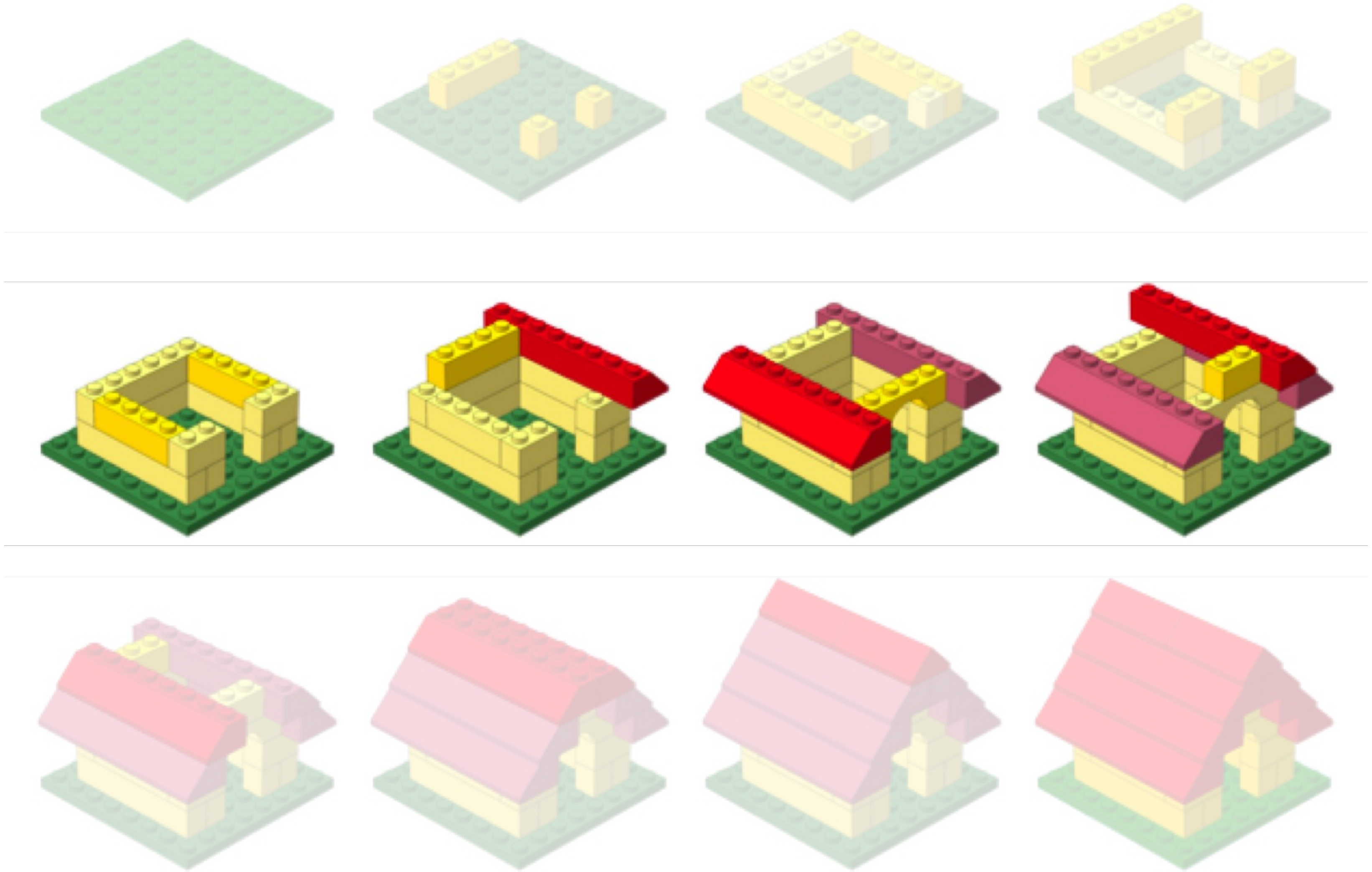
Setting up to perform Bioinformatics analysis

# 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
- Introduction to R
  - Parsing and working with smaller BED files
  - Statistical analysis, e.g. differential binding analysis
  - Generating figures from complex data

# Workshop scope



Bioinformatic Data Analysis

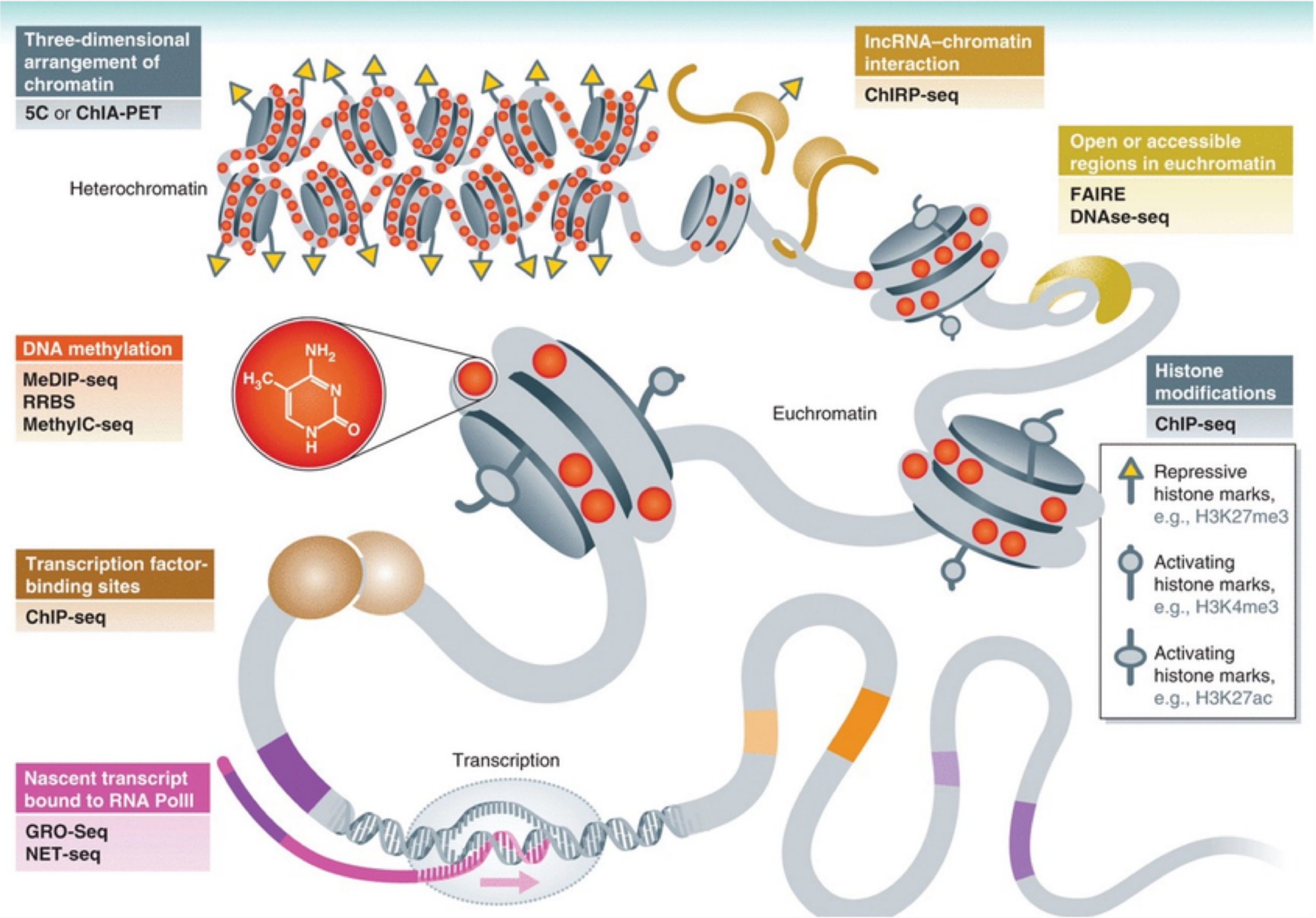
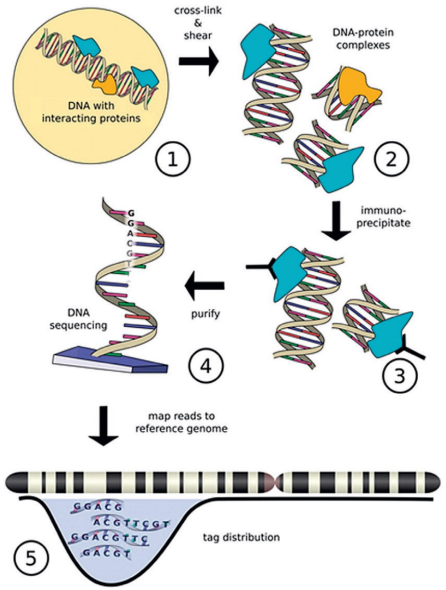

Three-dimensional arrangement of chromatin
5C or ChIA-PET
Heterochromatin
lncRNA–chromatin interaction
ChIRP-seq
Open or accessible regions in euchromatin
FAIRE
DNAse-seq
DNA methylation
MeDIP-seq
RRBS
MethylC-seq
NH2
H3C
N
O
N
H
Euchromatin
Histone modifications
ChIP-seq
Repressive histone marks, e.g., H3K27me3
Activating histone marks, e.g., H3K4me3
Activating histone marks, e.g., H3K27ac
Transcription factor-binding sites
ChIP-seq
Transcription
Nascent transcript bound to RNA PolII
GRO-Seq
NET-seq

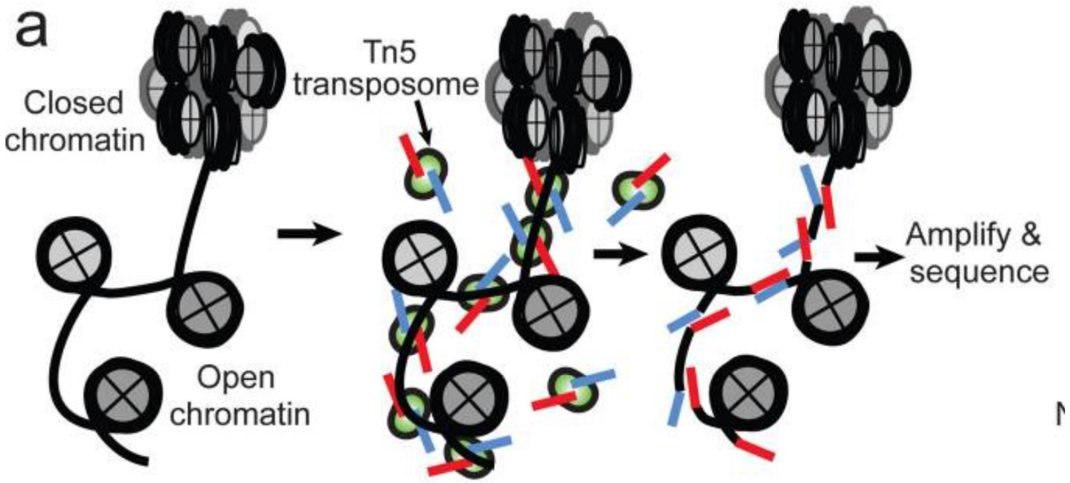
Figure adapted from Soon WW, Hariharan M, Snyder MP, “High throughput sequencing for biology and medicine”. Molecular Systems Biology 9:640 2013

# Genomic Methods for Profiling Chromatin

## ChIP-seq



## ATAC-seq



## CUT&RUN

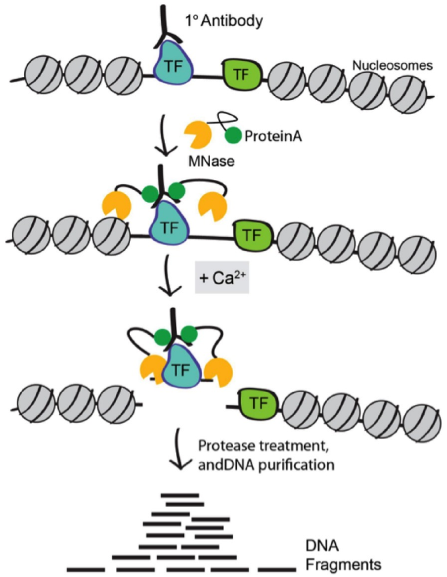
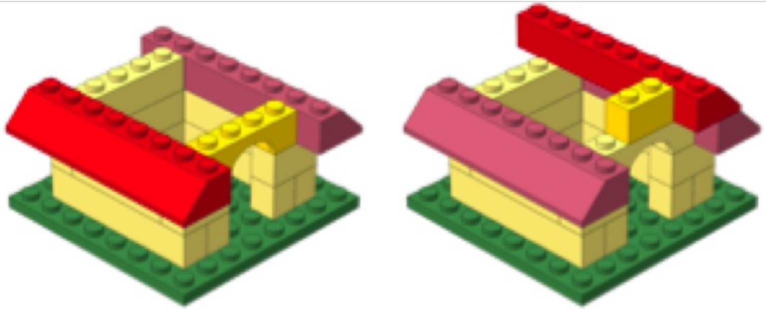


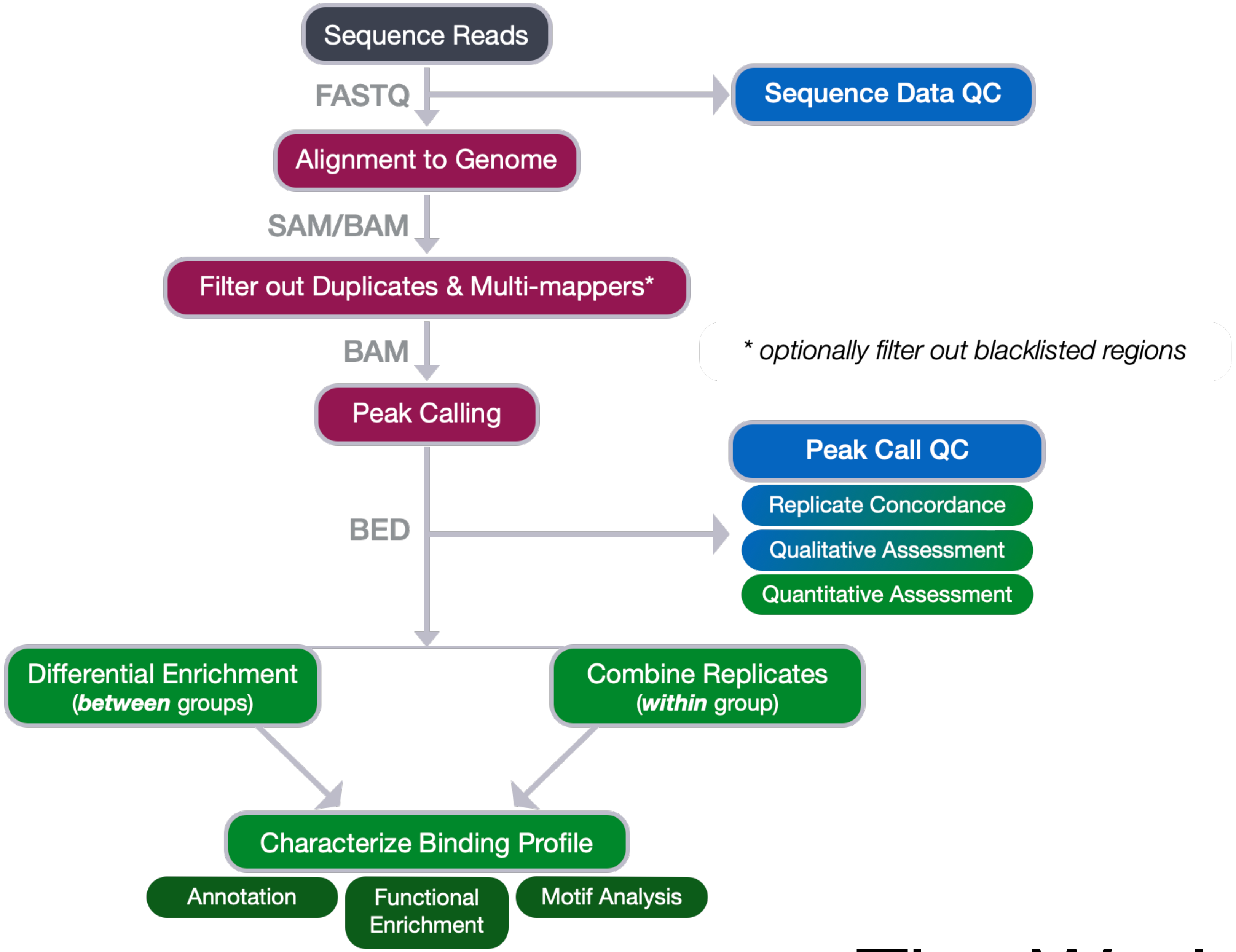
*Figure 1. CUT&RUN schematic (see text for details).*

# Learning Objectives



- Describe important considerations for setting up a successful ChIP-seq, CUT&RUN or ATAC-seq experiment
- Describe the steps in an ChIP-seq analysis workflow (from sequence data to peak calls) and contrast any differences for CUT&RUN and ATAC-seq analyses
- Learn how to handle various file formats encountered when analyzing ChIP-seq and related data
- Implement shell scripts on a high-performance compute cluster to perform the above steps

# The Workflow



Sequence Reads

FASTQ

Sequence Data QC

Alignment to Genome

SAM/BAM

Filter out Duplicates & Multi-mappers*

BAM

* *optionally filter out blacklisted regions*

Peak Calling

BED

Peak Call QC

Replicate Concordance

Qualitative Assessment

Quantitative Assessment

Differential Enrichment
(***between*** groups)

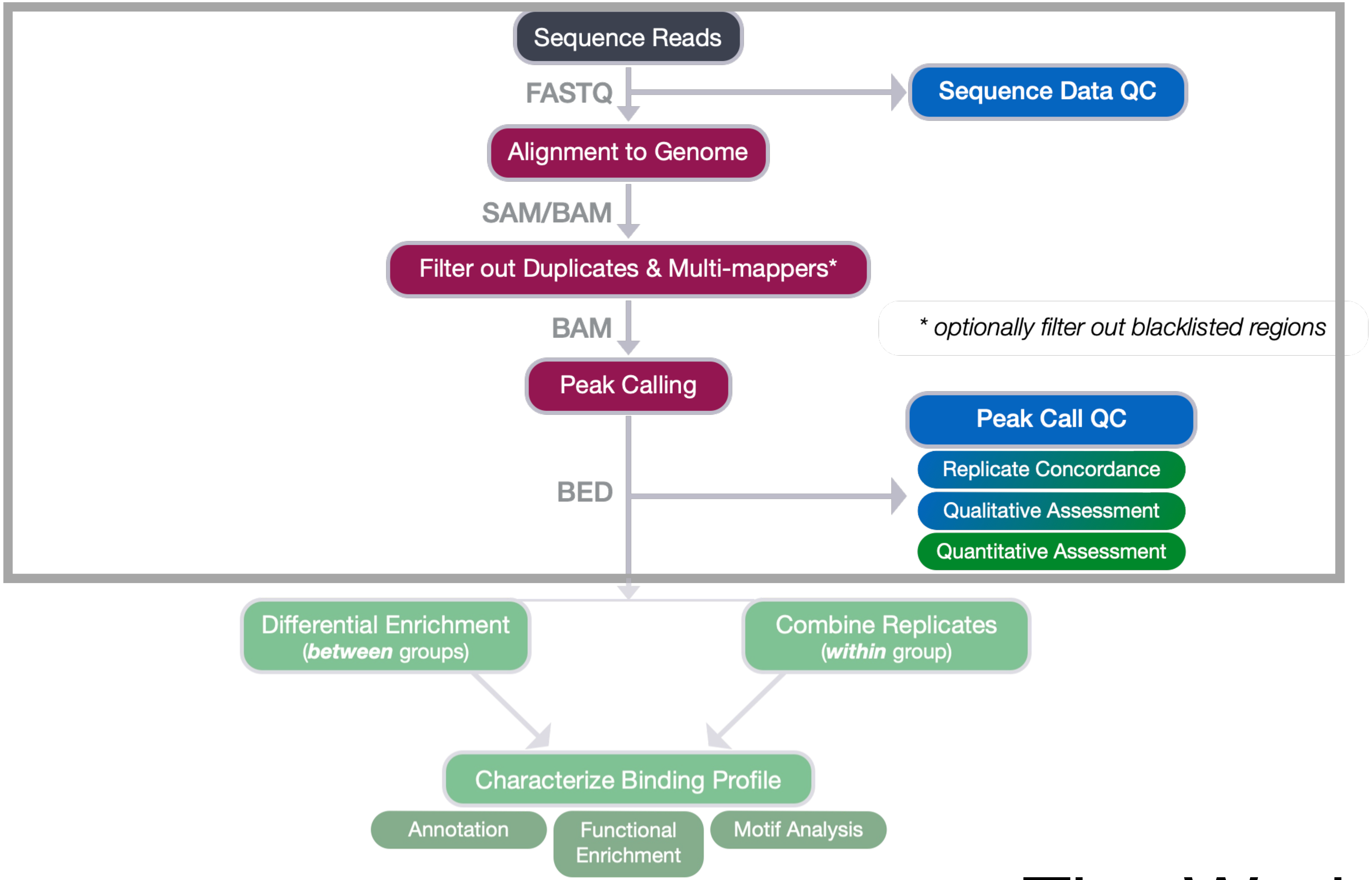
Combine Replicates
(***within*** group)

Characterize Binding Profile

Annotation

Functional Enrichment

Motif Analysis



# The Workflow

*Boxes in green represent parts of the workflow that will not be covered in this workshop*

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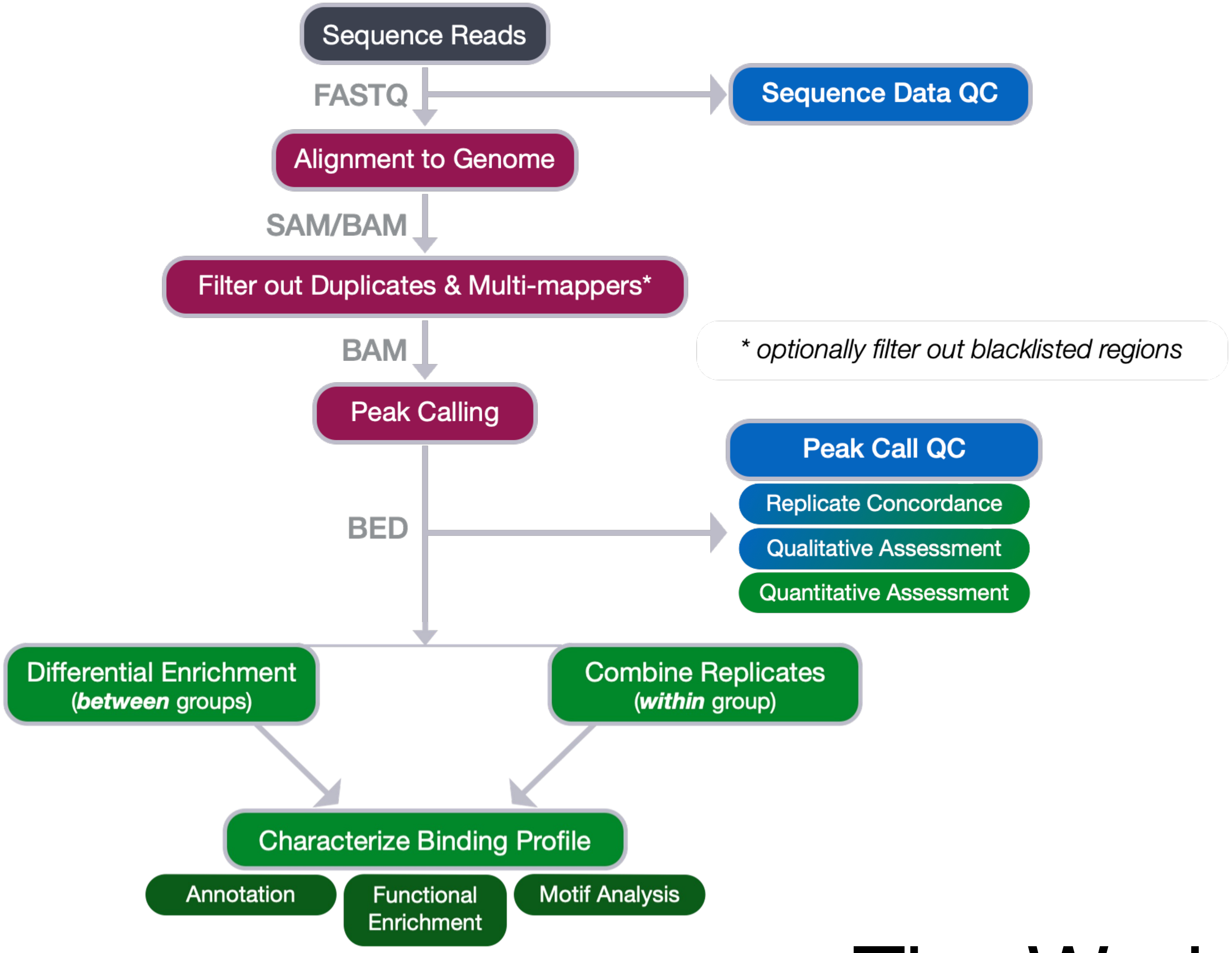

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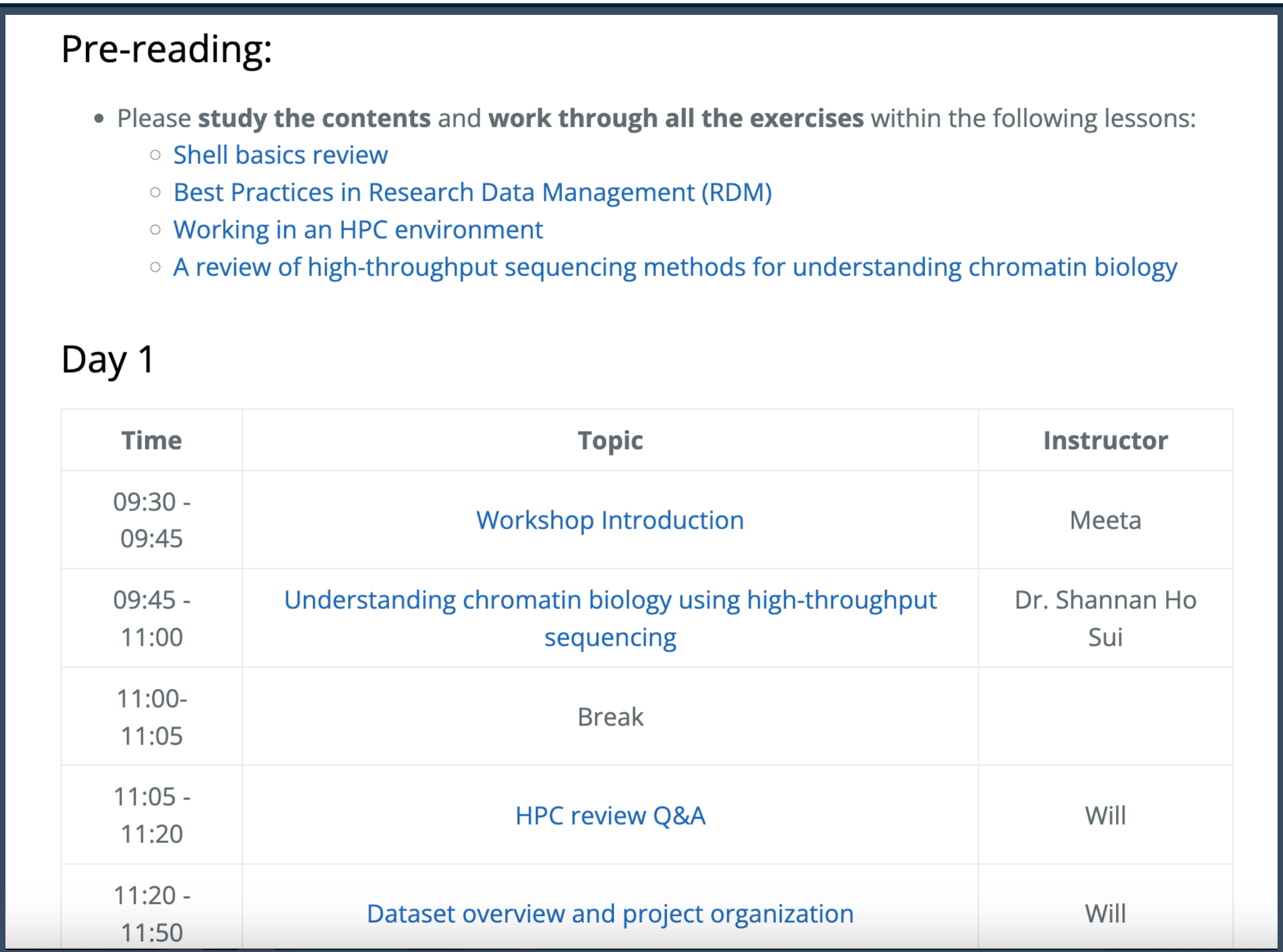
# Logistics

# Course schedule



## Pre-reading:

- Please **study the contents** and **work through all the exercises** within the following lessons:
  - Shell basics review
  - Best Practices in Research Data Management (RDM)
  - Working in an HPC environment
  - A review of high-throughput sequencing methods for understanding chromatin biology

## Day 1

| Time | Topic | Instructor |
|---|---|---|
| 09:30 - 09:45 | Workshop Introduction | Meeta |
| 09:45 - 11:00 | Understanding chromatin biology using high-throughput sequencing | Dr. Shannan Ho Sui |
| 11:00- 11:05 | Break | |
| 11:05 - 11:20 | HPC review Q&A | Will |
| 11:20 - 11:50 | Dataset overview and project organization | Will |

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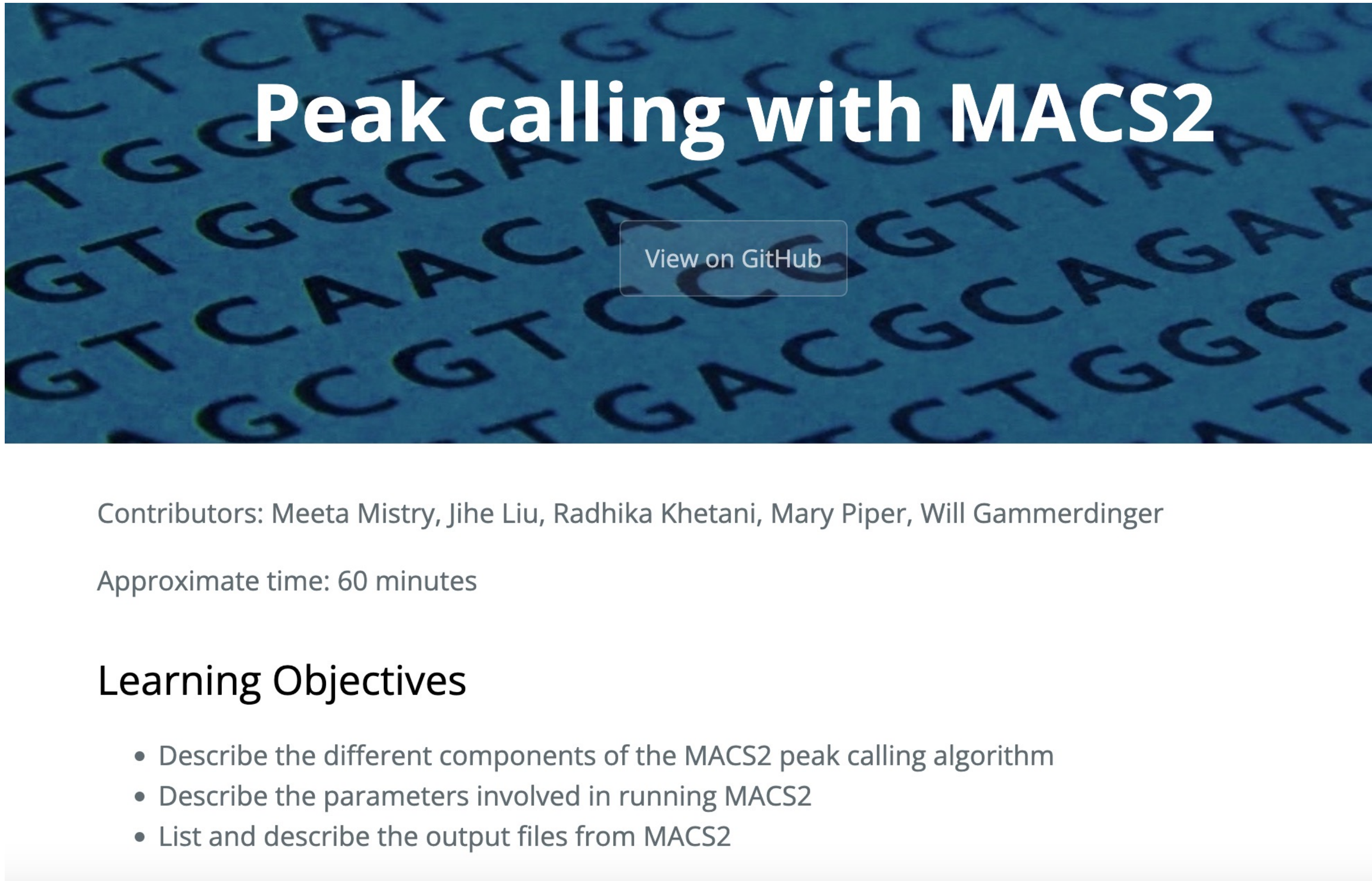
# Course materials

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



# Peak calling with MACS2

Contributors: Meeta Mistry, Jihe Liu, Radhika Khetani, Mary Piper, Will Gammerdinger

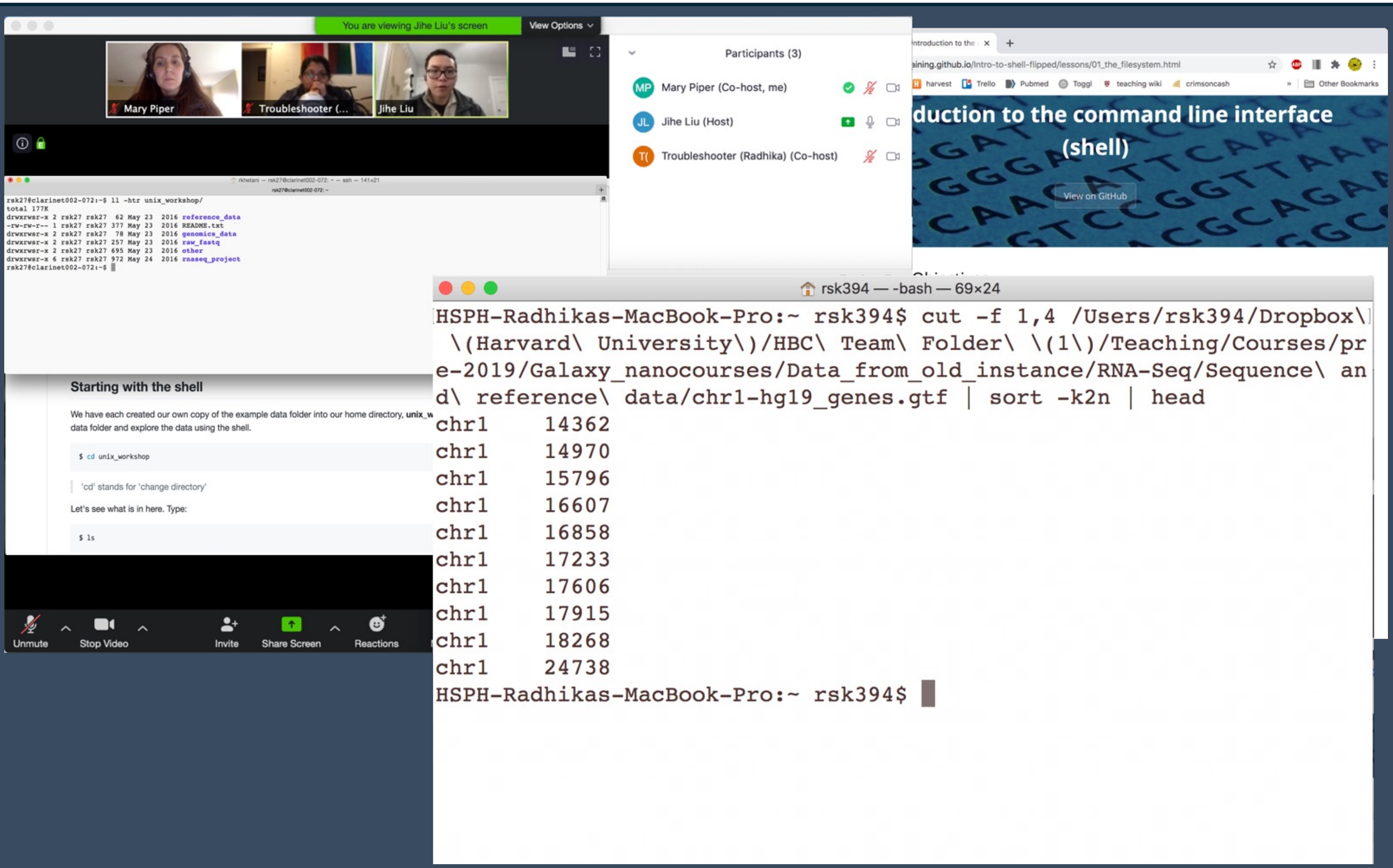
Approximate time: 60 minutes

## Learning Objectives

- Describe the different components of the MACS2 peak calling algorithm
- Describe the parameters involved in running MACS2
- List and describe the output files from MACS2

https://tinyurl.com/hbc-chipseq

# Single Screen & 3 Windows



```
rsk27@clarinet002-072:~$ ll -htr unix_workshop/
total 177K
drwxrwsr-x 2 rsk27 rsk27  62 May 23  2016 reference_data
-rw-rw-r-- 1 rsk27 rsk27 377 May 23  2016 README.txt
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drwxrwsr-x 6 rsk27 rsk27 972 May 24  2016 rnaseq_project
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```

### 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
```

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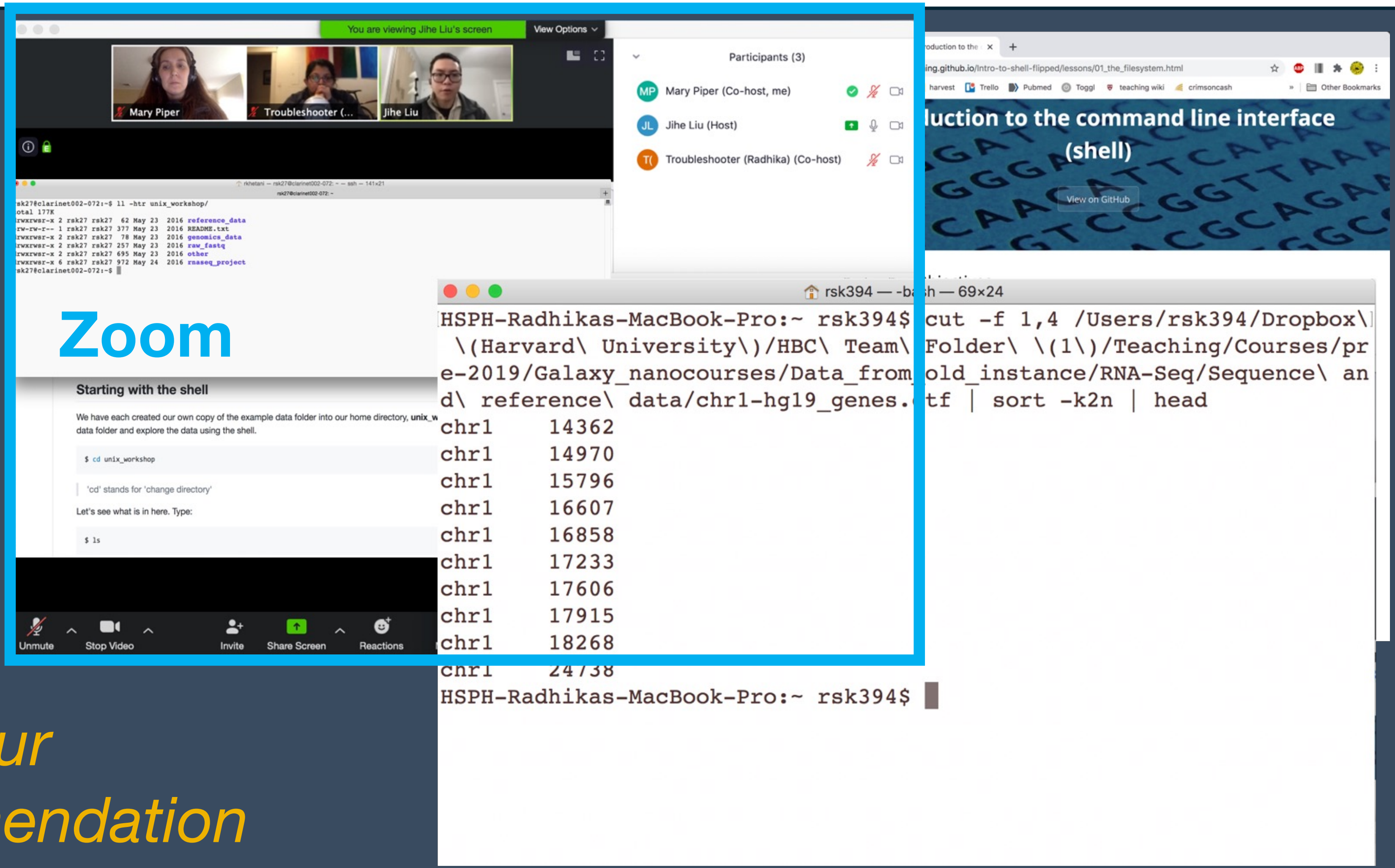

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*Our Recommendation*

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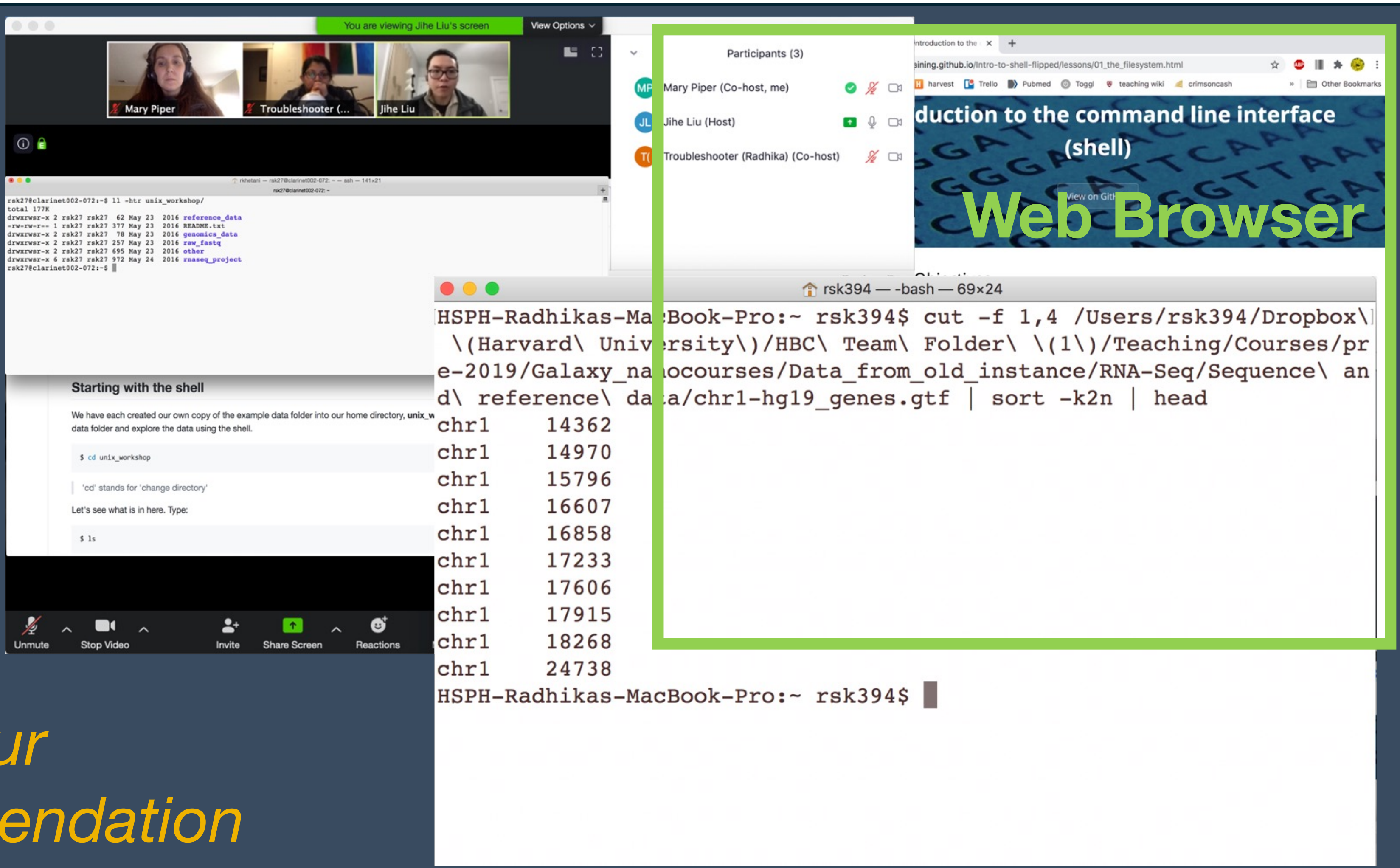

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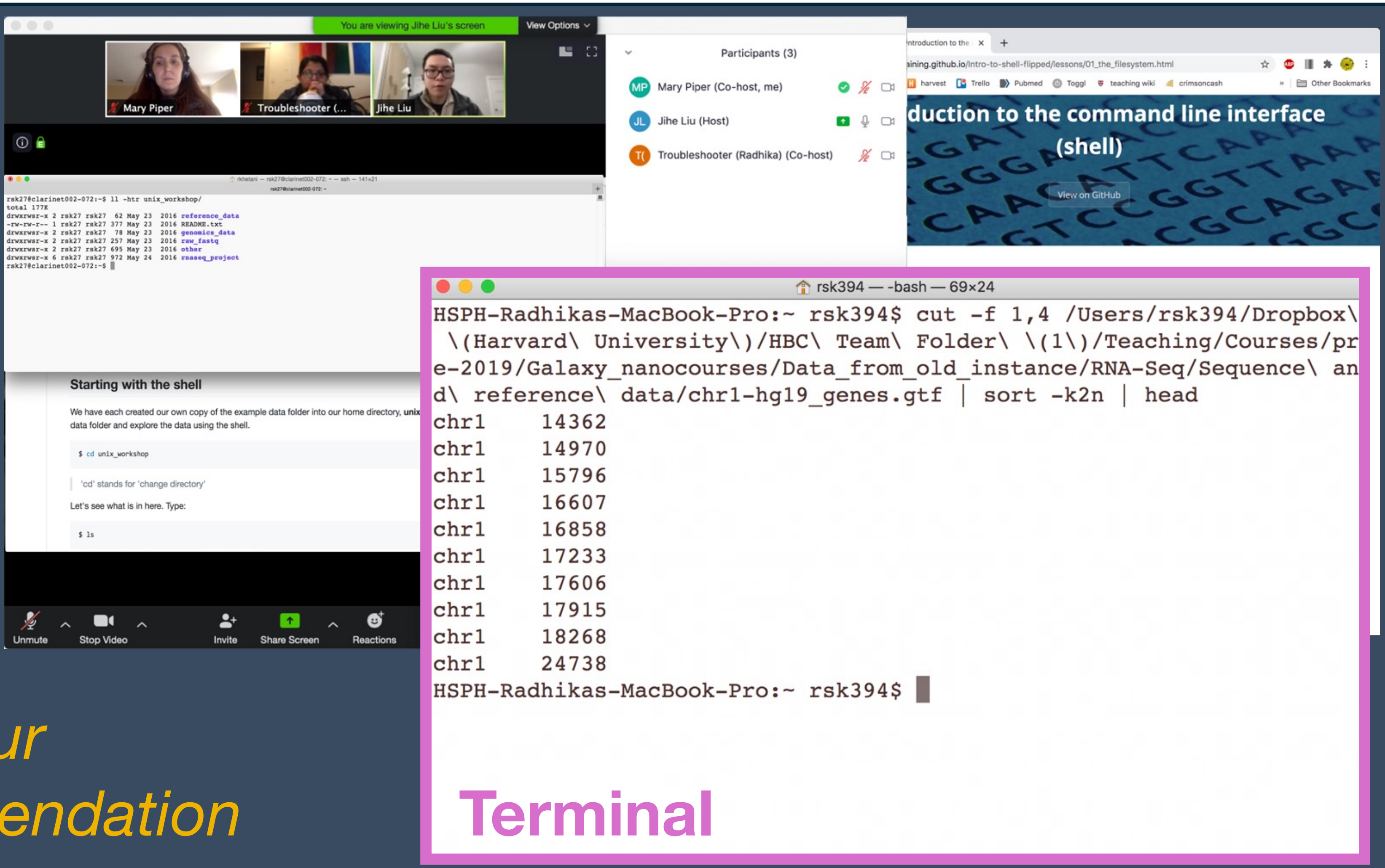

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*Our Recommendation*

Terminal

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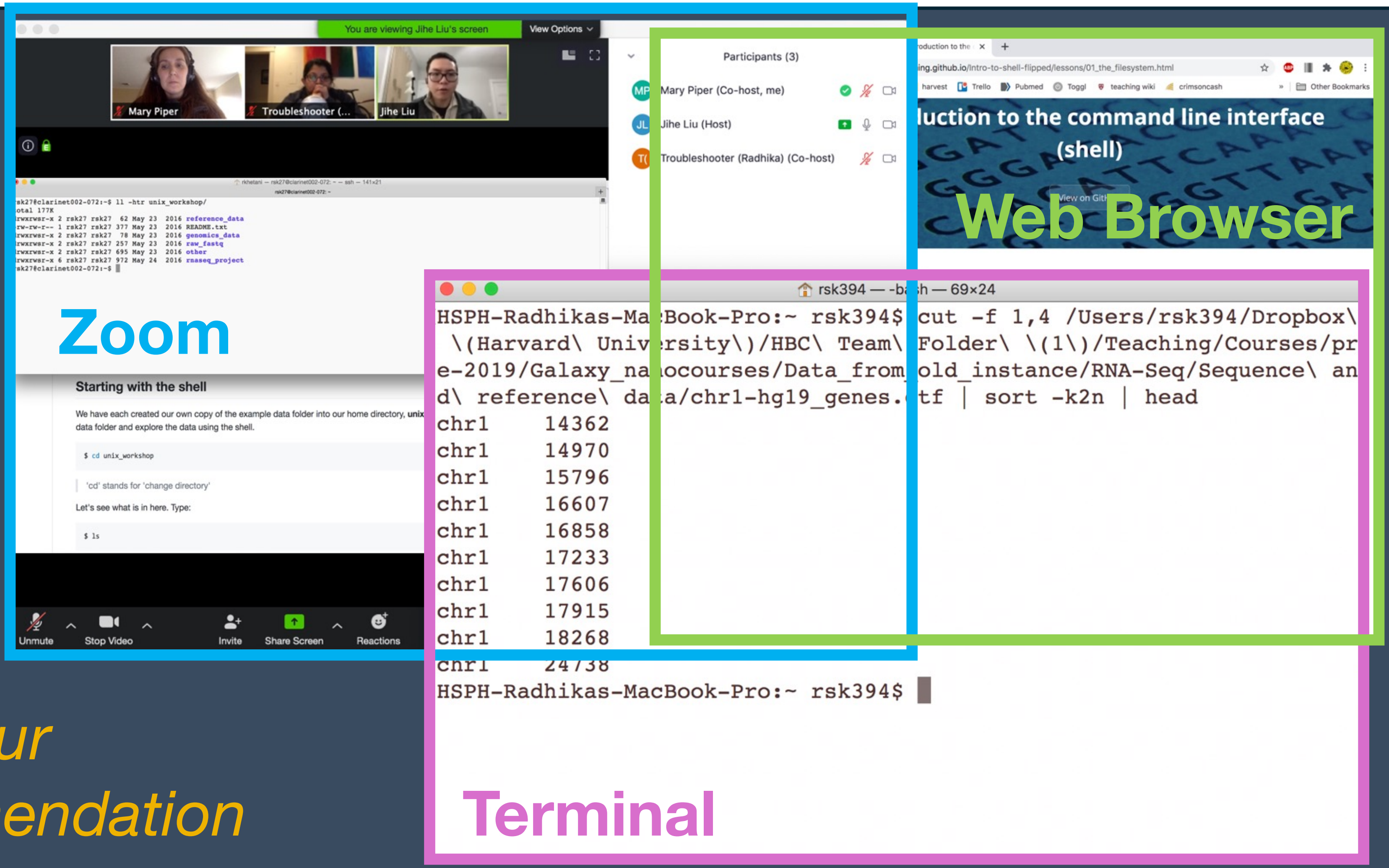

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```

*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

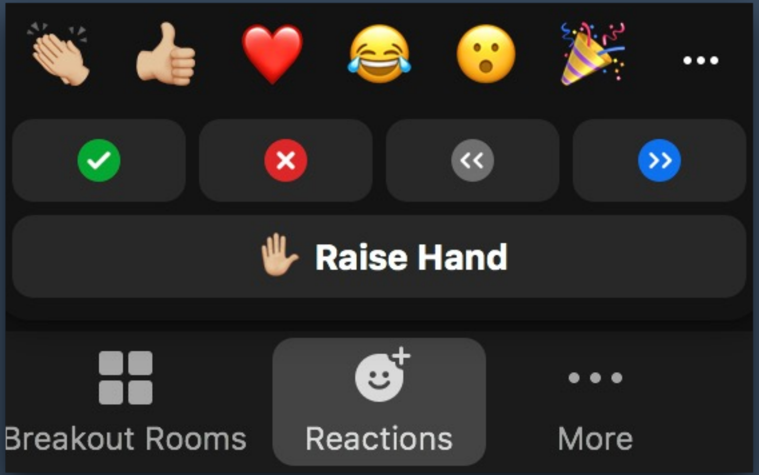
- At-home lessons and exercises after each session
- Cover material not previously discussed
- Provides us feedback to help pace the course appropriately
- 3-5 hours to complete
- Homework load is heavier in the beginning of this workshop series and tapers off

# Using AI for Assignments

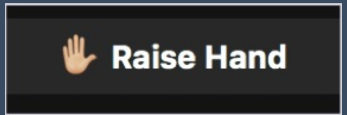
- Do
  - Try to resolve error messages with it
  - Test code written by AI on a dataset where you have expected results
  - Take the time to review the generated code line-by-line
- Don’t
  - Implement it in replacement to learning
  - Write code that you don’t understand
  - Assume the output from an 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?
  - Post the question in the Chat window OR
  -  when the presenter asks for questions

  - Let the Moderator know

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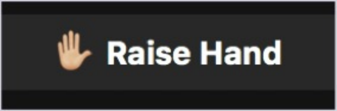
  - 

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

# Thanks!

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

# Contact Us

HBC
Harvard Chan Bioinformatics Core

- *HBC training team:* hbctraining@hsph.harvard.edu
- *HBC consulting:* bioinformatics@hsph.harvard.edu
- *O2 (HMS-RC):* rchelp@hms.harvard.edu