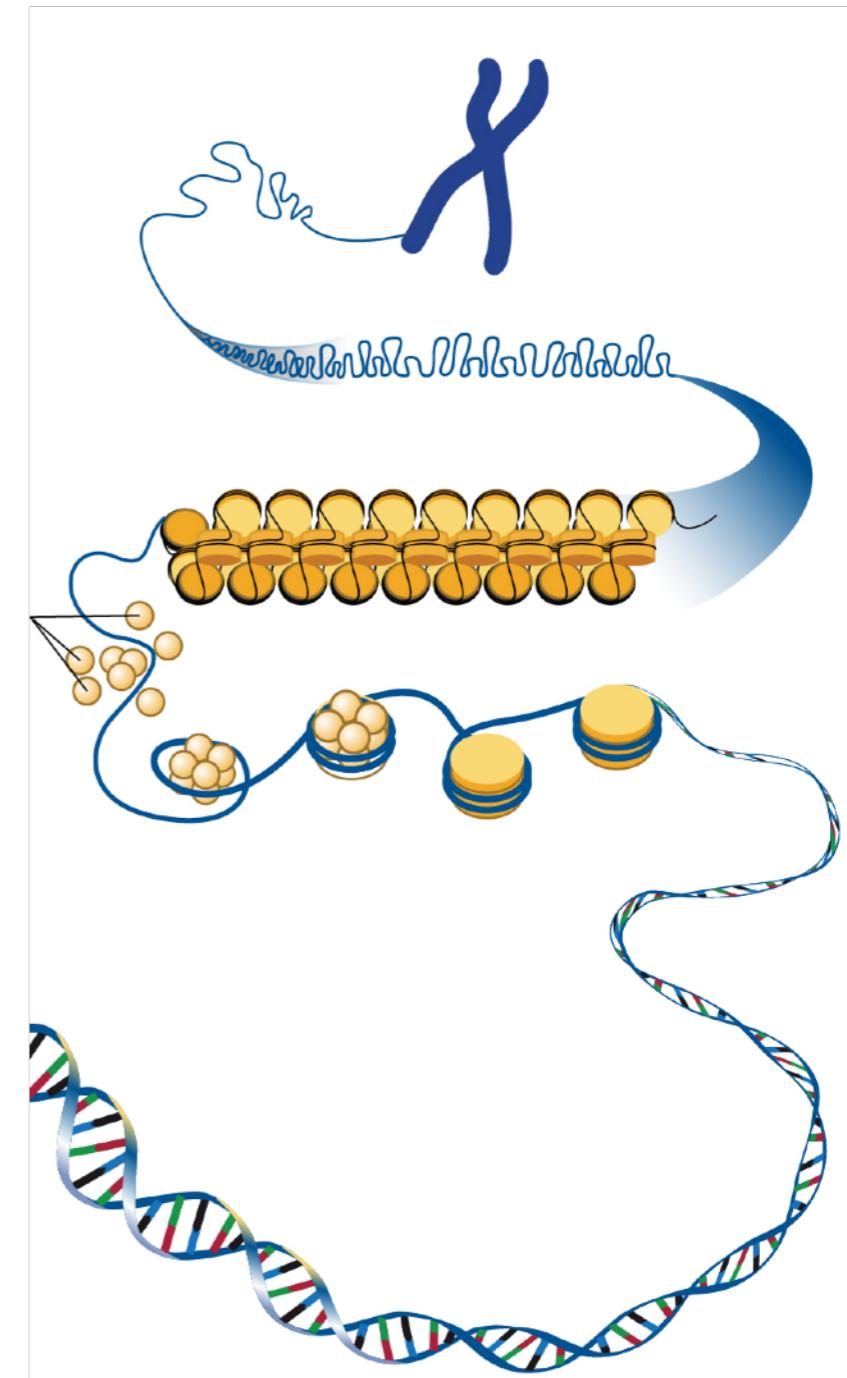


# Understanding chromatin biology using high throughput sequencing methods

Harvard Chan Bioinformatics Core  
in collaboration with  
HMS Research Computing

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





Shannan Ho Sui  
*Director*



Victor Barrera



Amelie Jule



Zhu Zhuo



Radhika Khetani  
*Director of Education*



Meeta Mistry  
*Interim Director  
of Education*



Heather Wick



Will Gammerdinger



Emma Berdan



Sergey Naumenko



Maria Simoneau



Noor Sohail



James Billingsley

# 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/>



**HARVARD**  
**T.H. CHAN**  
SCHOOL OF PUBLIC HEALTH

NIEHS

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Maria Simoneau



Noor Sohail



James Billingsley

# 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.*

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

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

# Training

A key component of the HBC's mission is to train researchers at Harvard and beyond.

HBC's training team is made up of scientists, educators, and community based organizations who work on research projects to ensure the best training for our students.

Our hands-on workshops are designed to provide an emphasis on **experimental design** and **informatics** for **wet-lab biologists** and **bioinformaticians** alike.

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 workshop is designed for those with no prior experience in bioinformatics.



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**DF/HCC**  
DANA-FARBER / HARVARD CANCER CENTER



THE HARVARD CLINICAL  
AND TRANSLATIONAL  
SCIENCE CENTER



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

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

The workshops focus on the analysis of high-throughput sequencing data, with an emphasis on **experimental design**, **informatics**, and **reproducibility**. Our workshops are designed to teach the skills needed for performing wet-lab experiments and analysing the resulting sequencing data.

**bioinformatics)**

**NGS) data\*\***

and **bioinformatics** 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 provide training for researchers at Harvard and beyond.

HBC's training team is made up of experts in training and community building who work on research projects to ensure the quality of our training.

Our hands-on workshops are designed with an emphasis on **experimental design** and **informatics**, for **wet-lab biologists** and **bioinformaticians** alike.

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 workshop is currently available online.



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THE HARVARD CLINICAL  
AND TRANSLATIONAL  
SCIENCE CENTER

**HARVARD  
MEDICAL SCHOOL**

Our dedicated training team holds workshops to help researchers learn how to analyze and interpret 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**, **informatics**, and **reproducibility**. Our workshops are designed to help researchers understand the principles of sequencing-based experiments and analysing the resulting data.

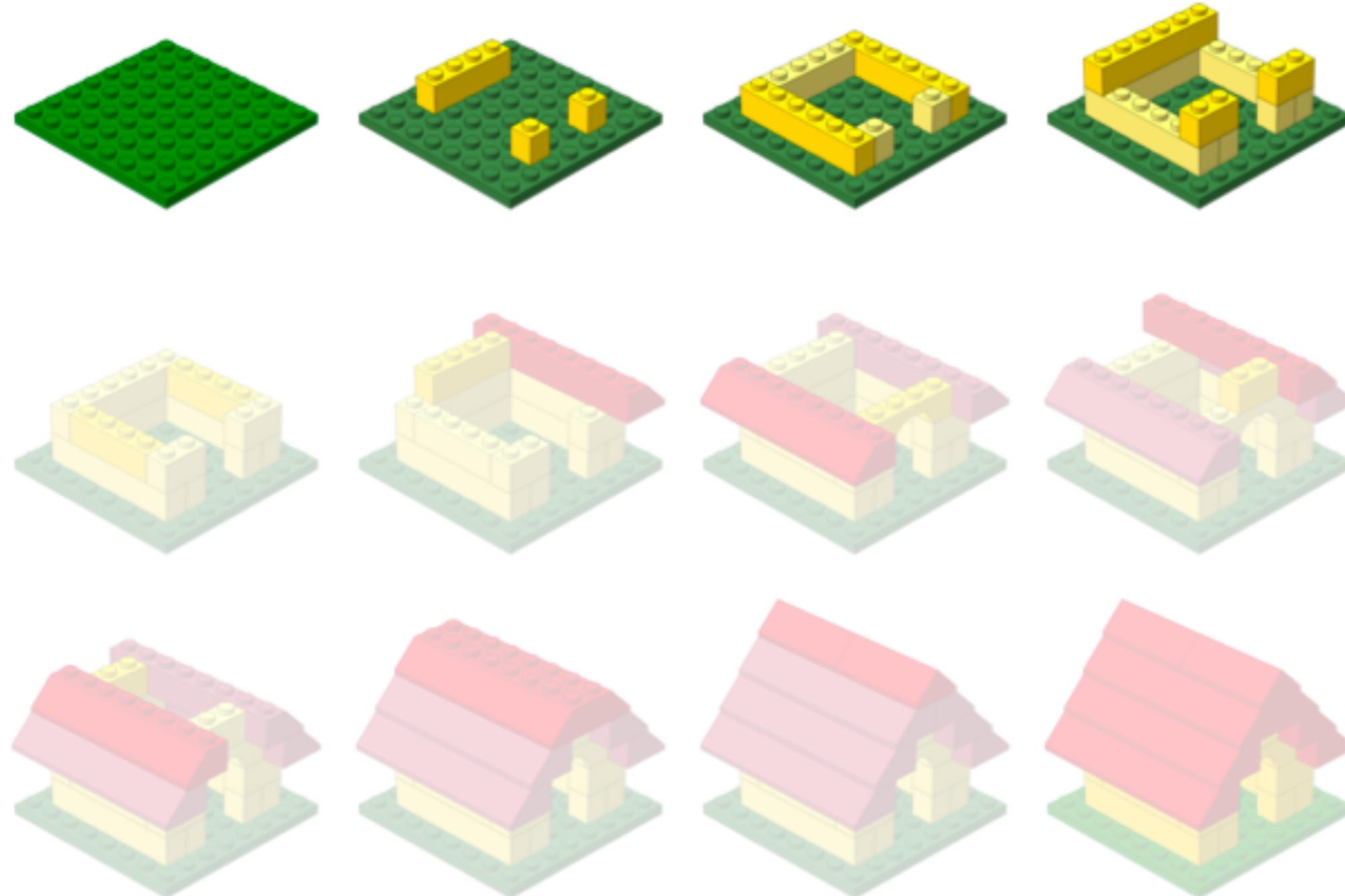
**informatics)**

**NGS) data\*\***

and **reproducibility** for the advanced workshops.

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

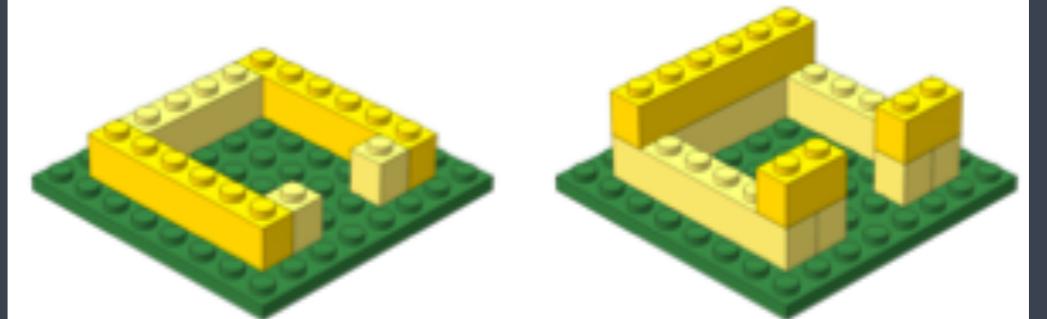
<https://hbctraining.github.io/main/>



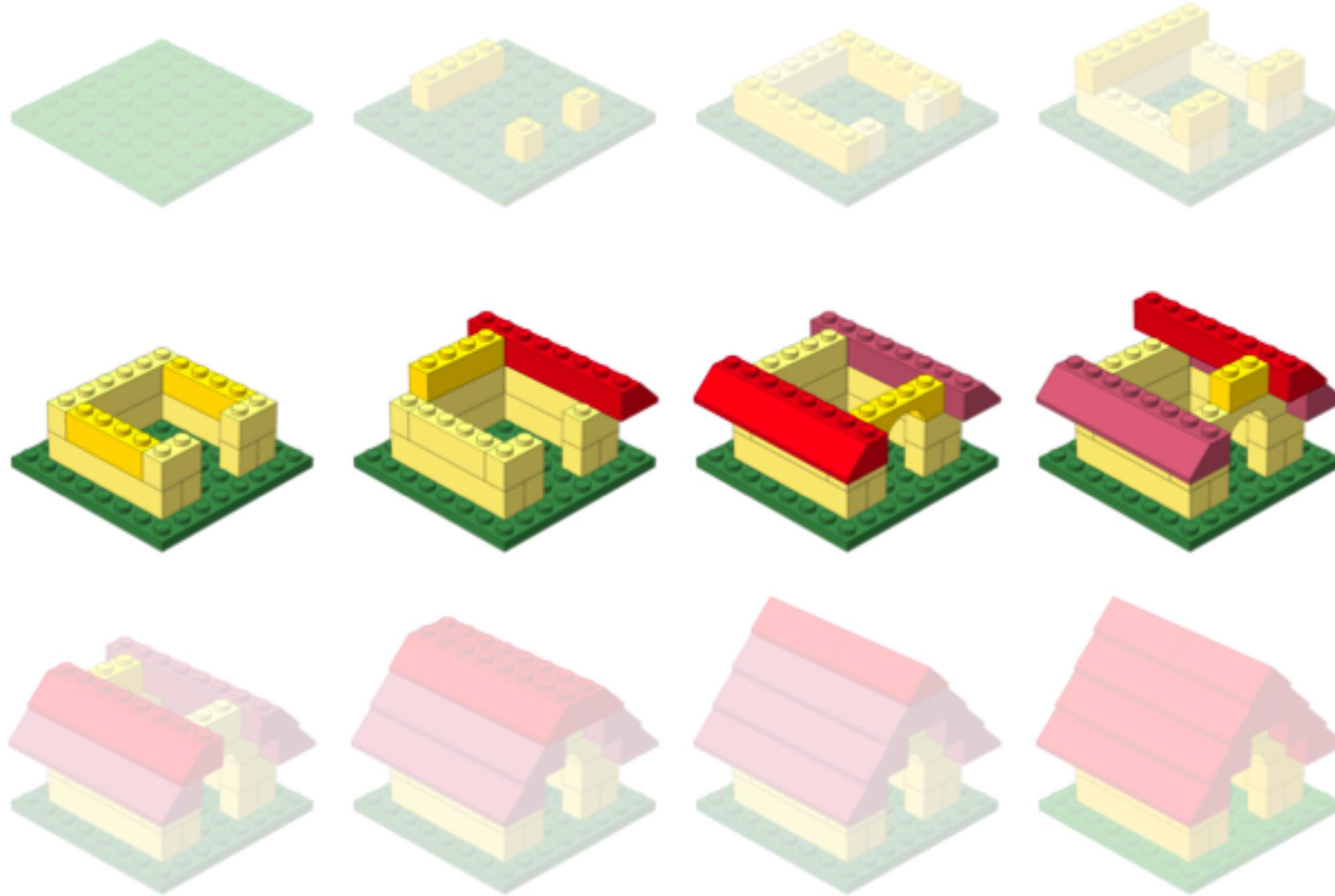
<http://anoved.net/tag/lego/page/3/>

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 BED files
  - Statistical analysis, e.g. differential binding analysis
  - Generating figures from complex data



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

Bioinformatics data analysis

# Workshop scope

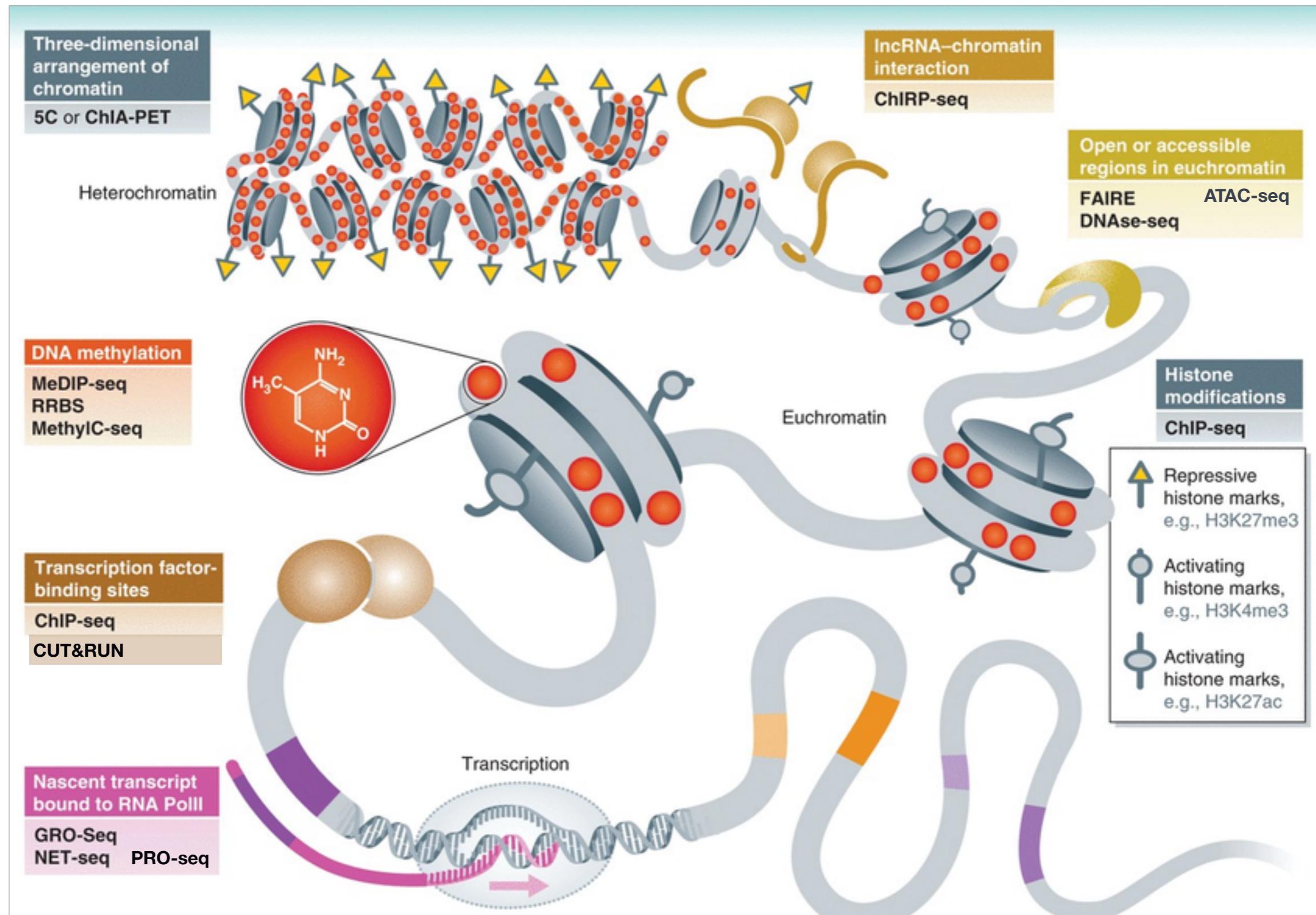
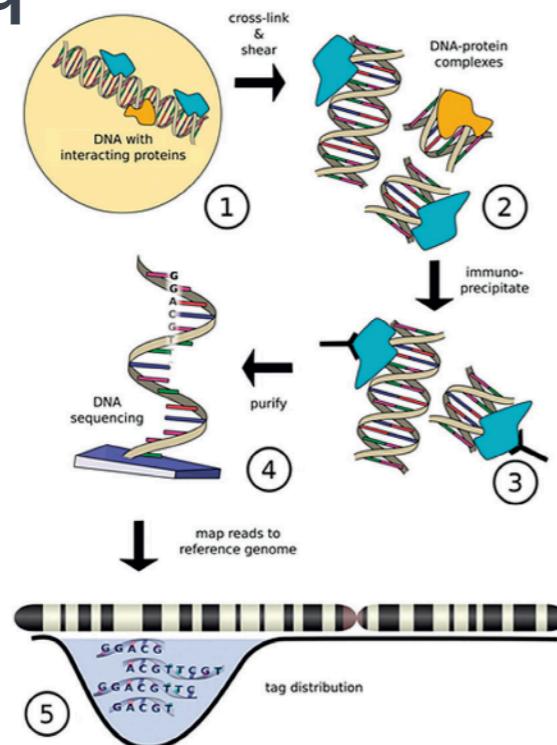


Figure adapted from Soon WW, Hariharan M, Snyder MP, "High throughput sequencing for biology and medicine". Molecular Systems Biology 9:640 2013

# Genomic methods for profiling chromatin

## ChIP-seq



## CUT&RUN

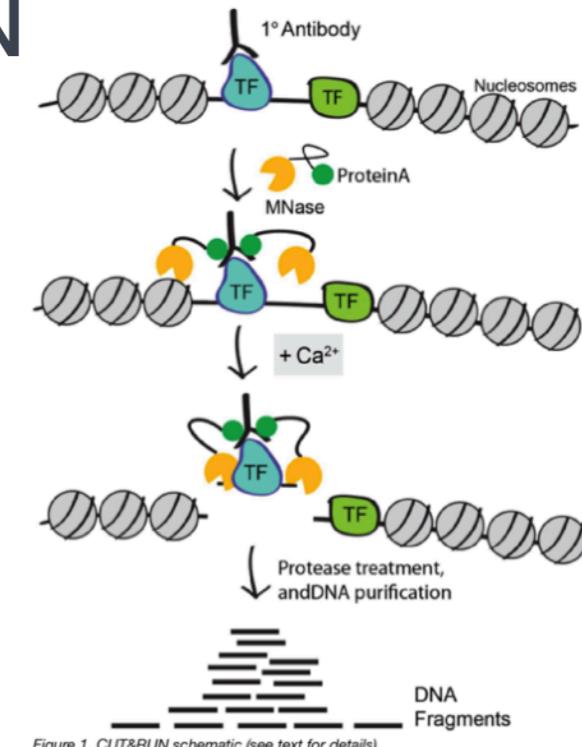
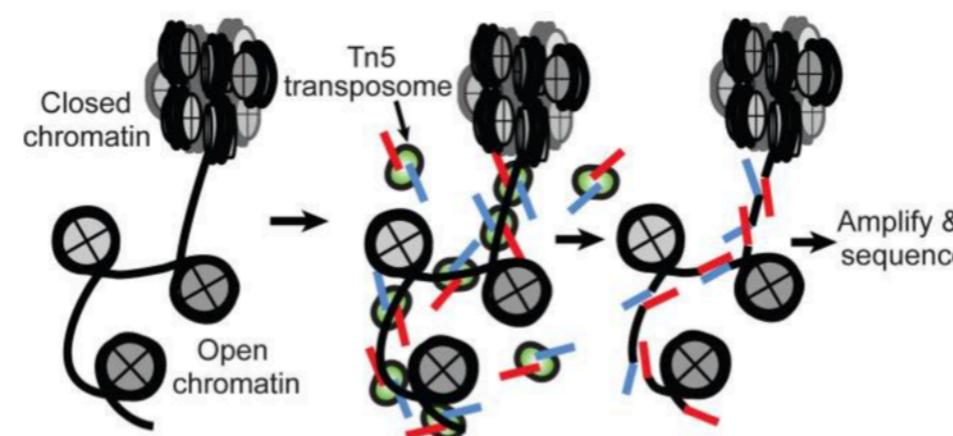
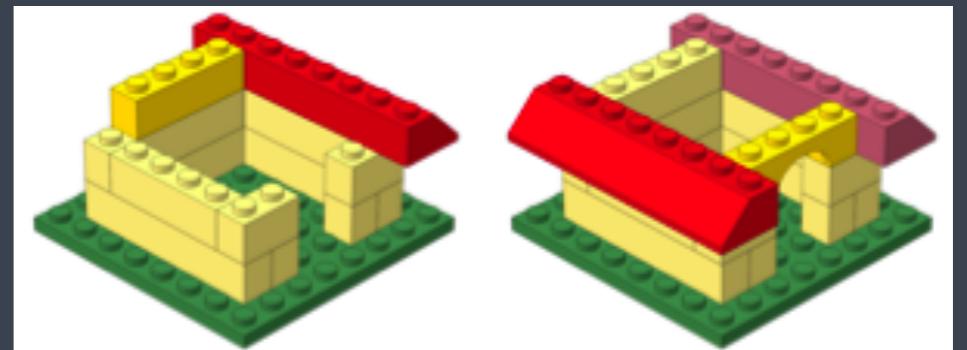


Figure 1. CUT&RUN schematic (see text for details).

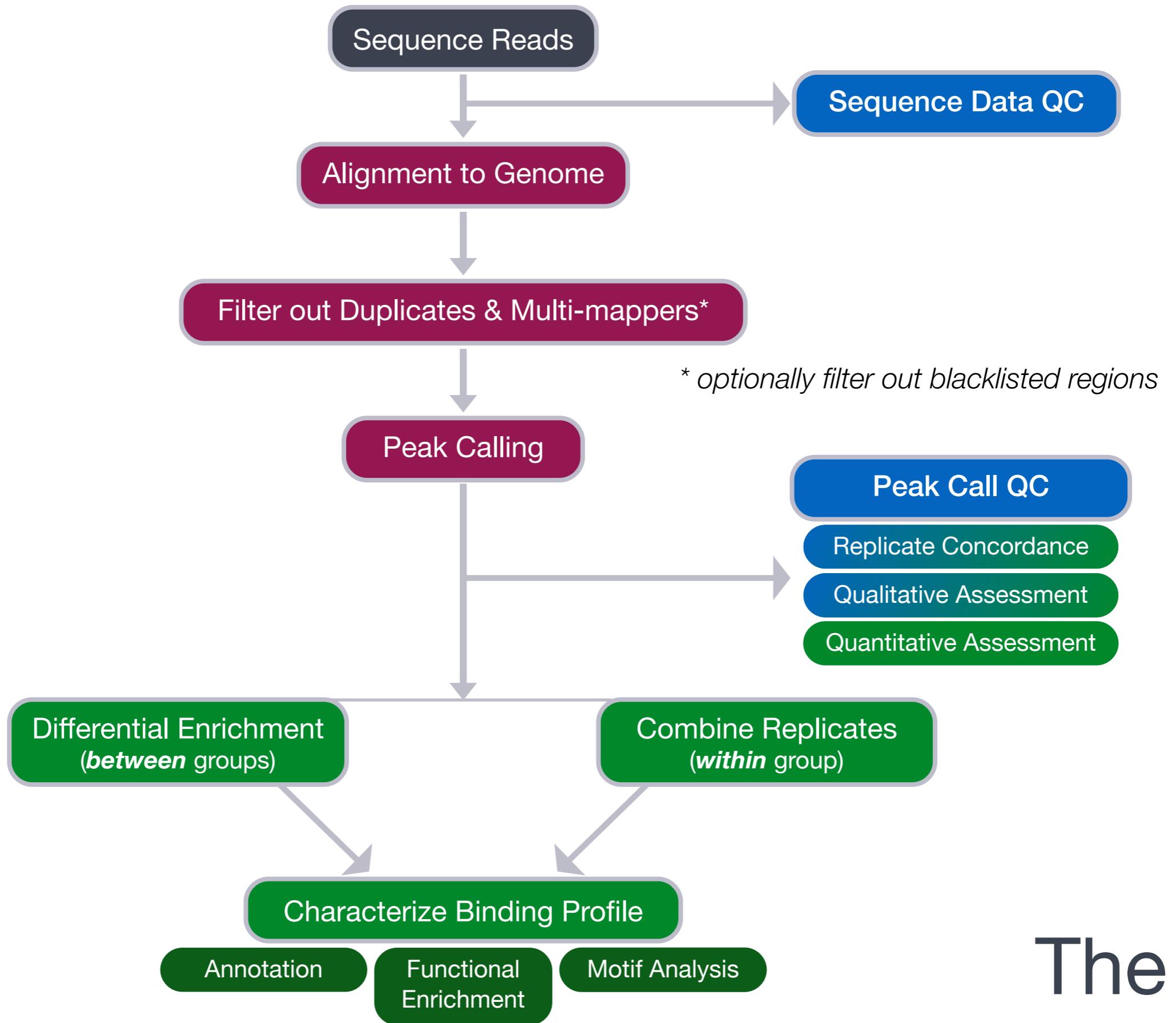
## ATAC-seq



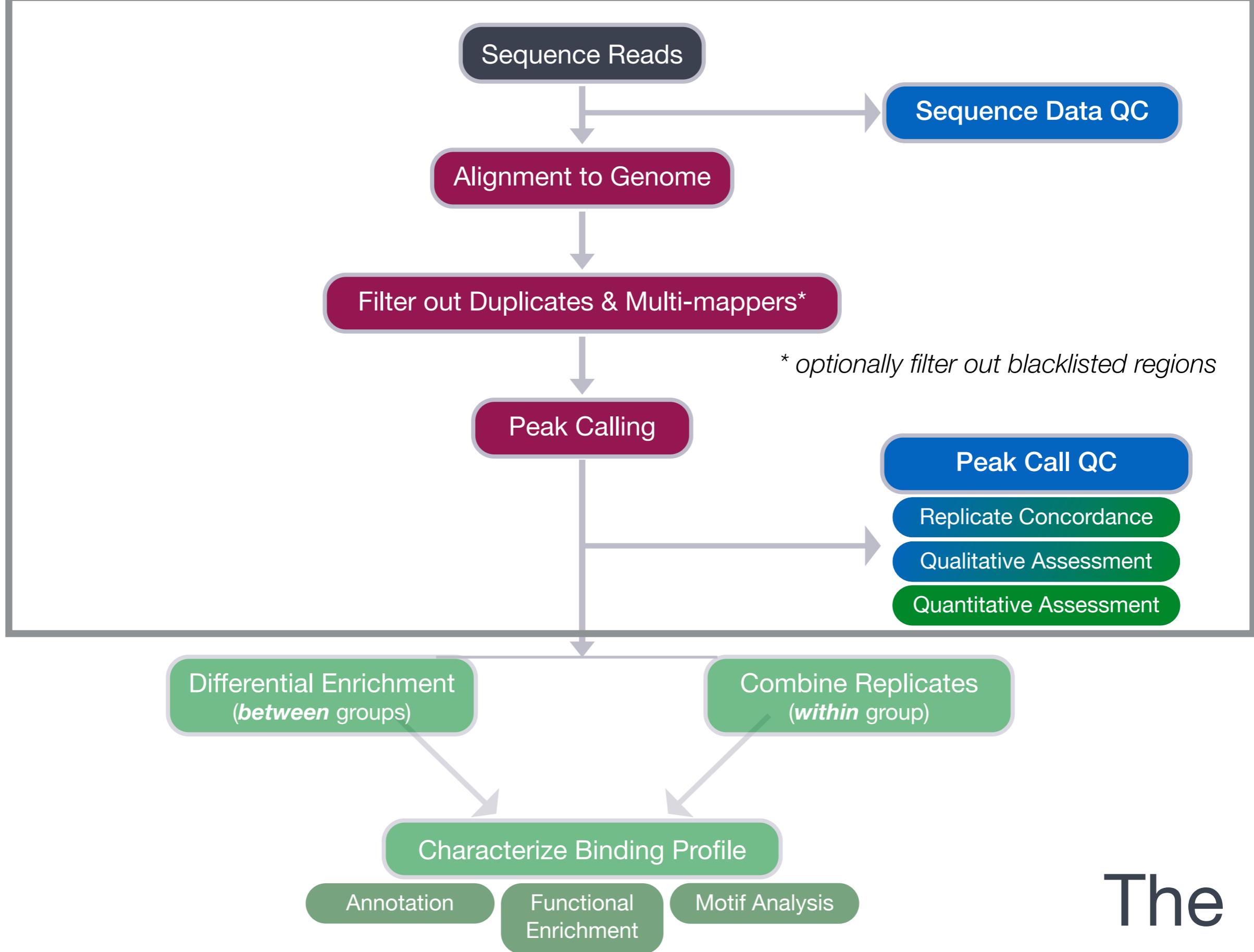
# Learning Objectives



- ✓ 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

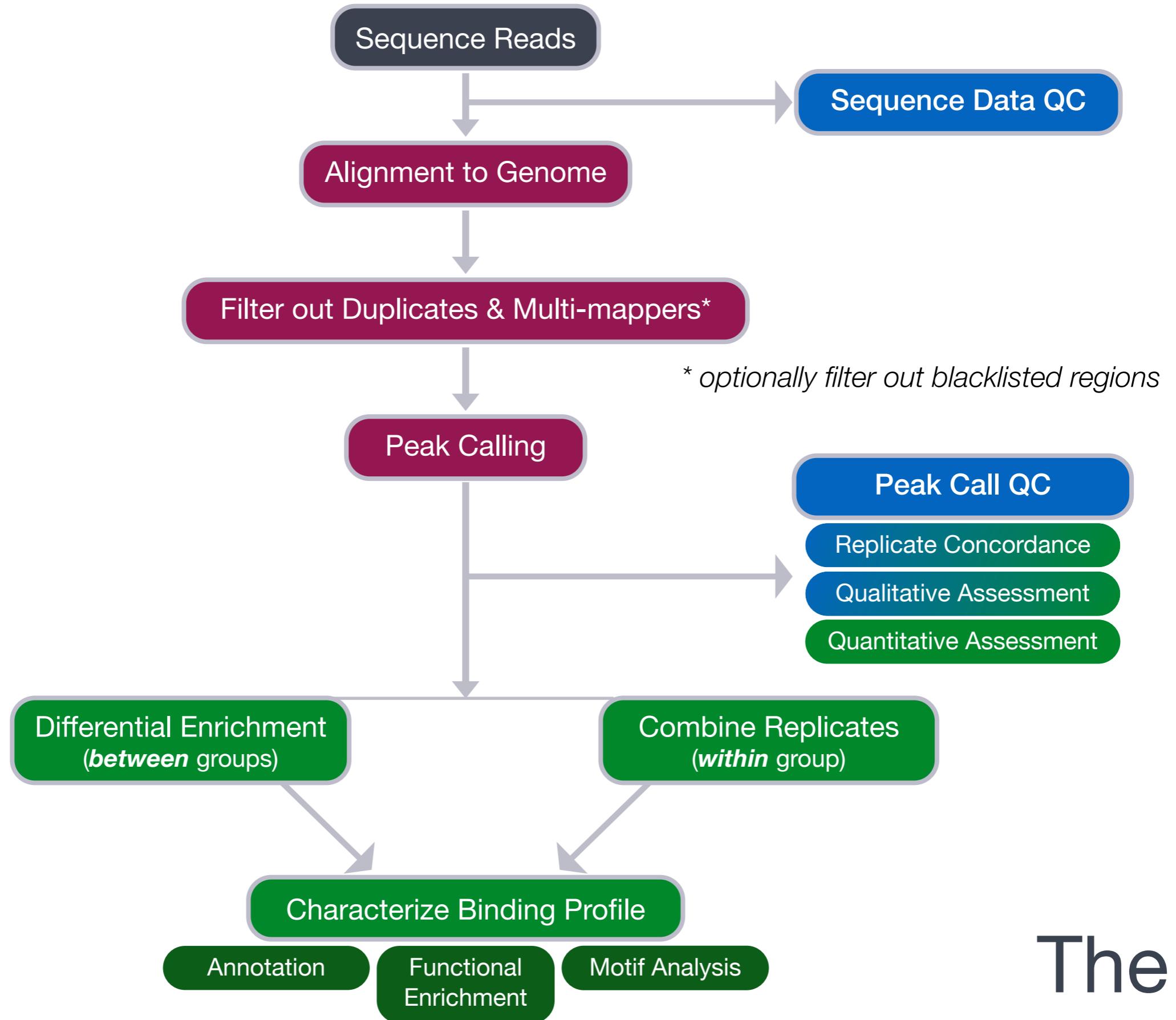


# The Workflow



# The Workflow

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



# The Workflow

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

# Logistics

# Course webpage

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

# Course schedule online

## Workshop Schedule

**NOTE:** The [Basic Data Skills Introduction to the command-line interface](#) workshop is a prerequisite.

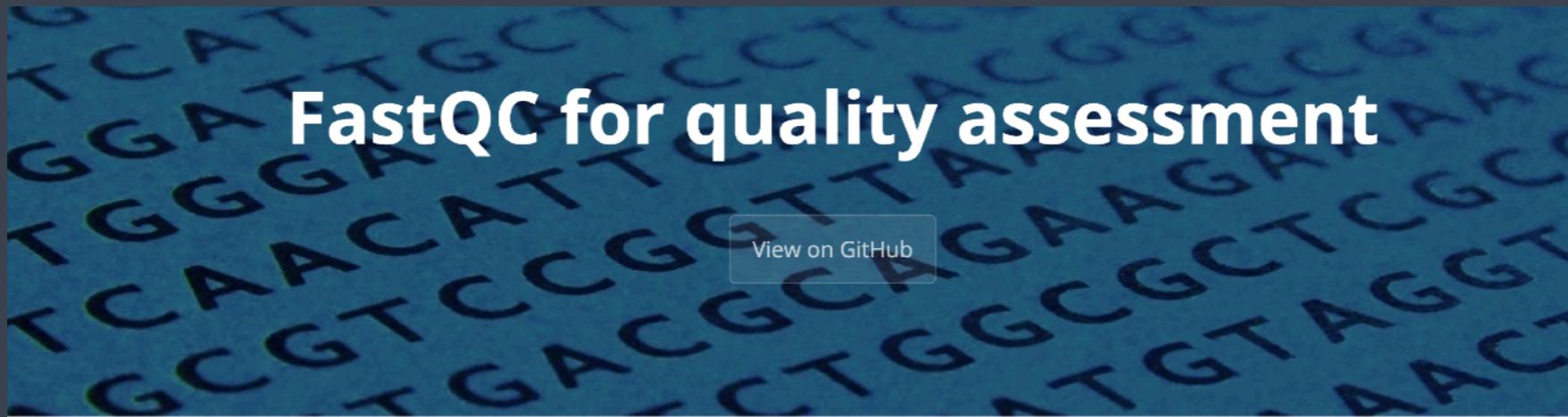
### Pre-reading:

- 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	Jihe

# Course materials online



## FastQC for quality assessment

[View on GitHub](#)

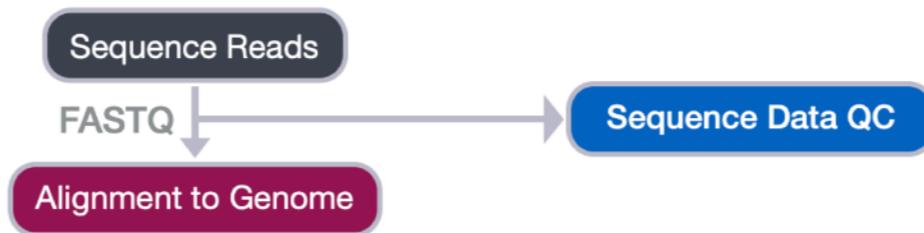
Contributors: Mary Piper, Radhika Khetani, Jihe Liu, Will Gammerdinger

Approximate time:

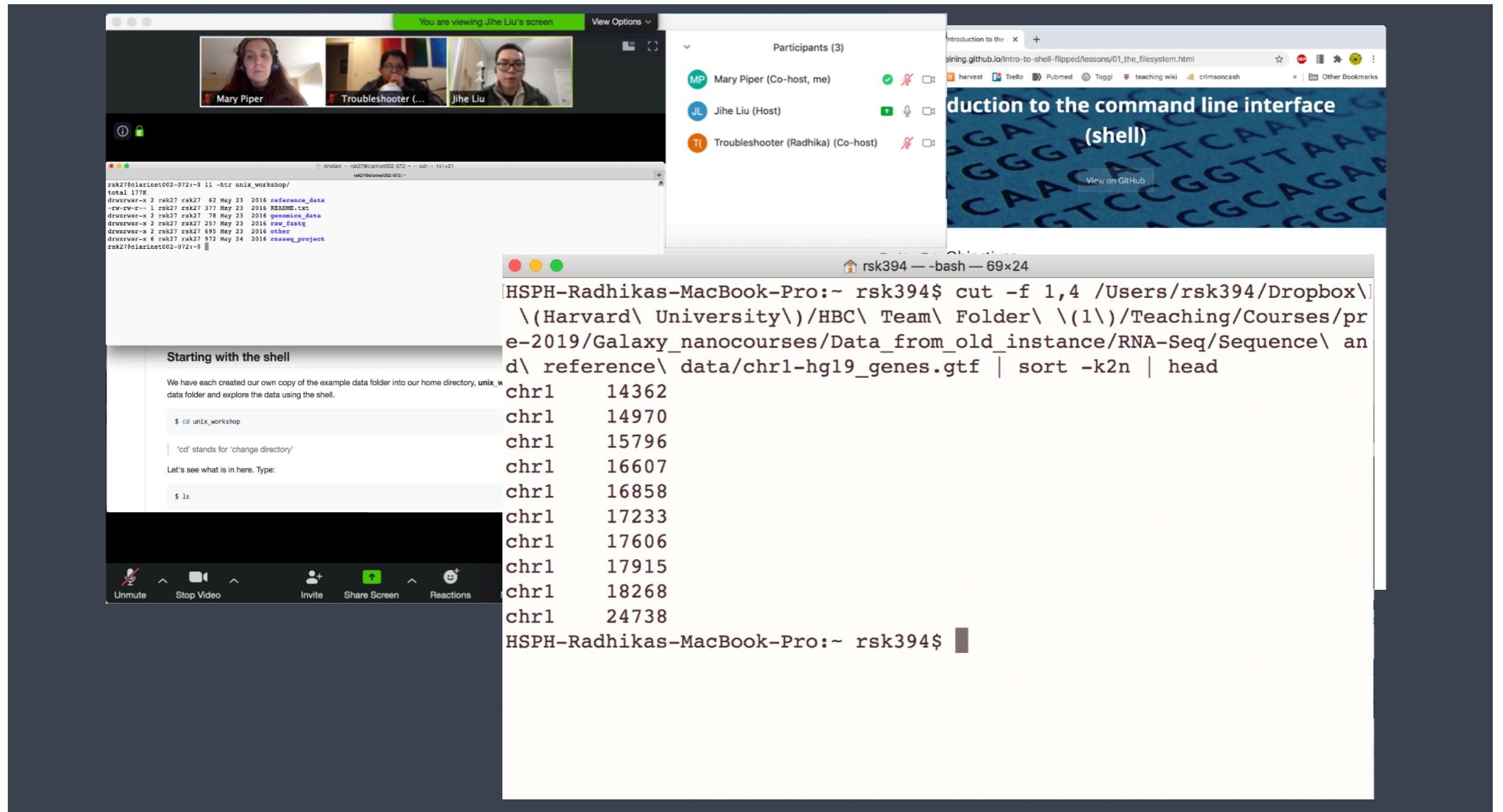
### Learning Objectives

- Explain the components of a FASTQ file
- Evaluate the quality of the sequencing data using FastQC

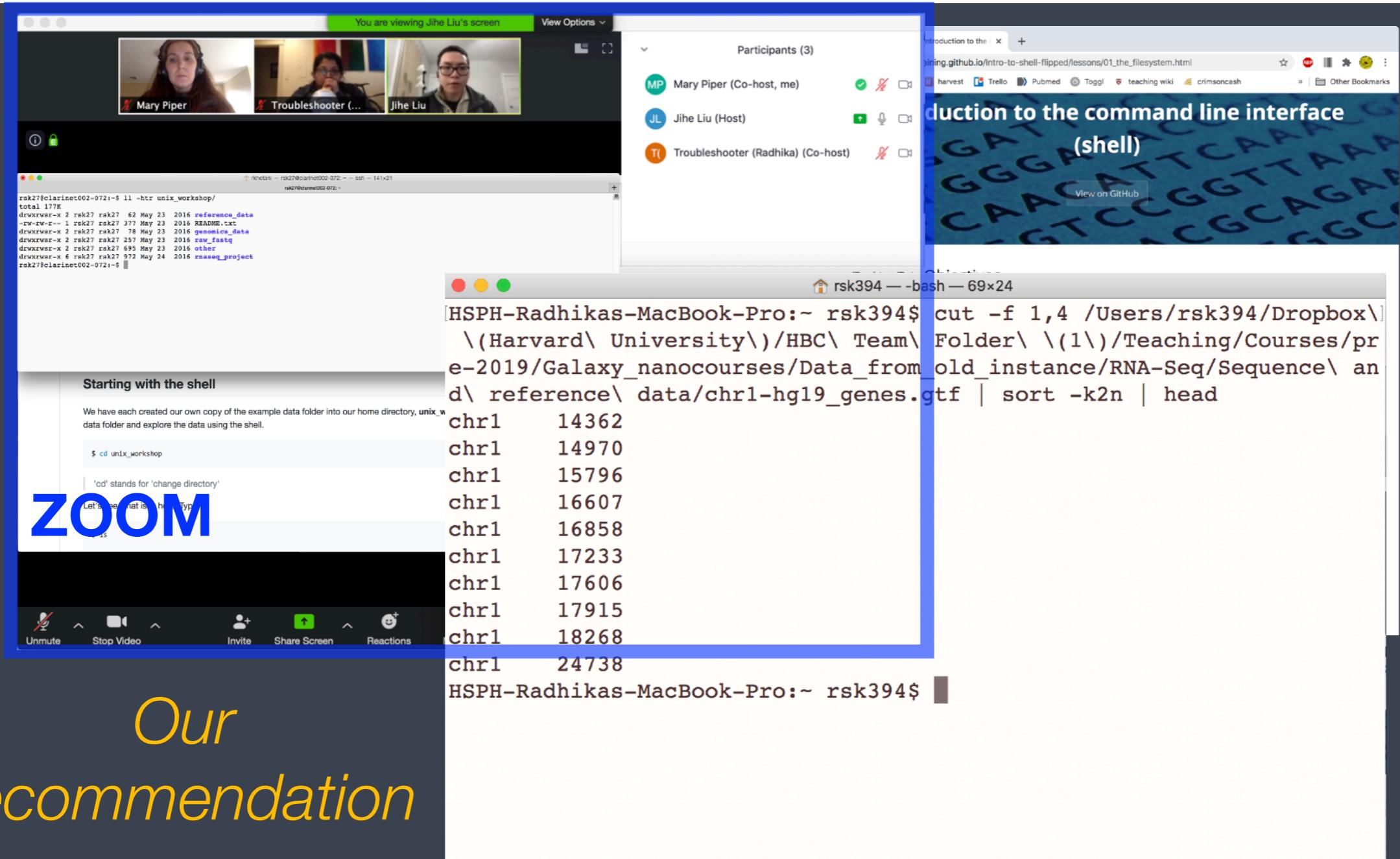
### Quality control of sequence reads



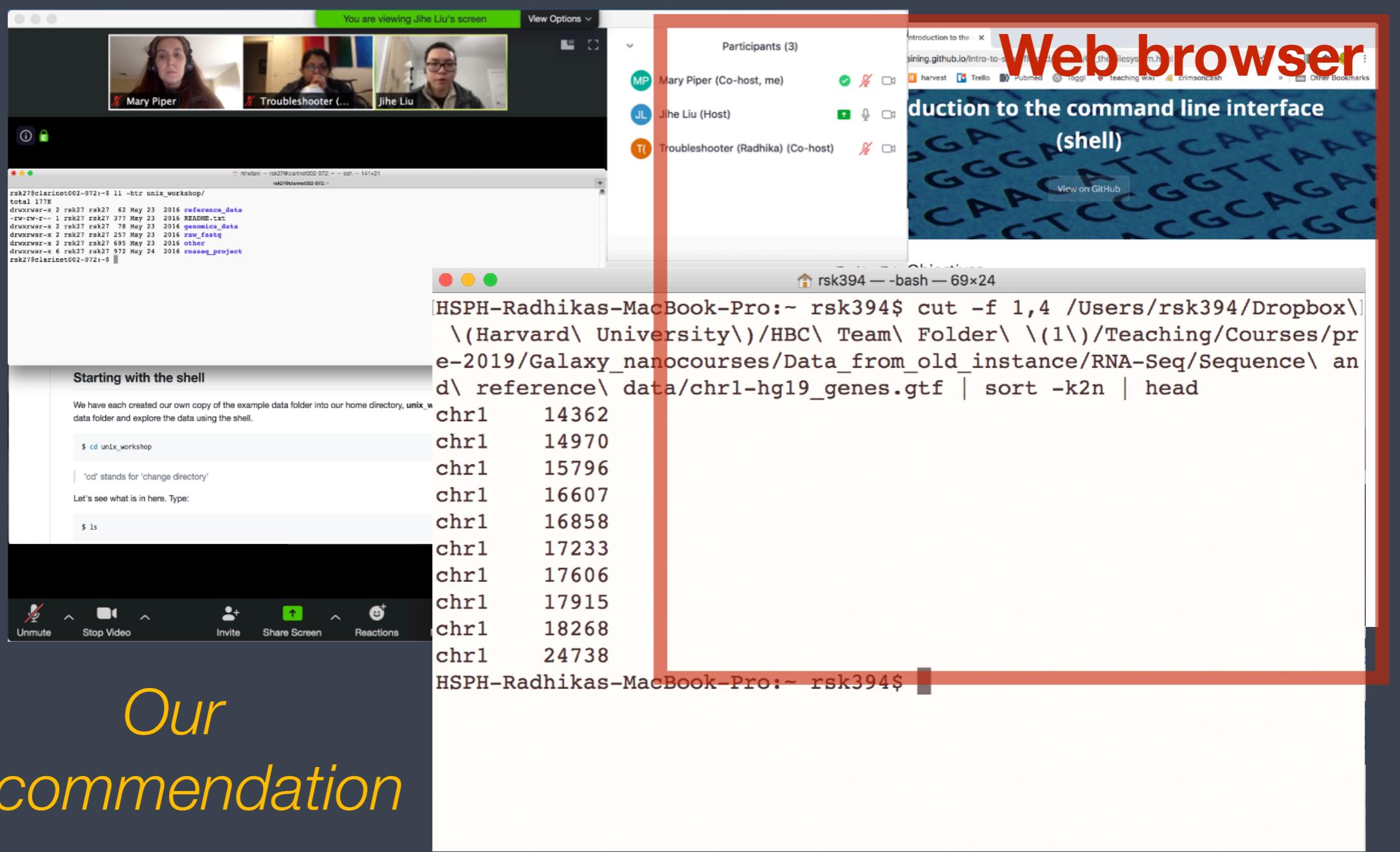
# Single screen & 3 windows?



# Single screen & 3 windows?



# Single screen & 3 windows?



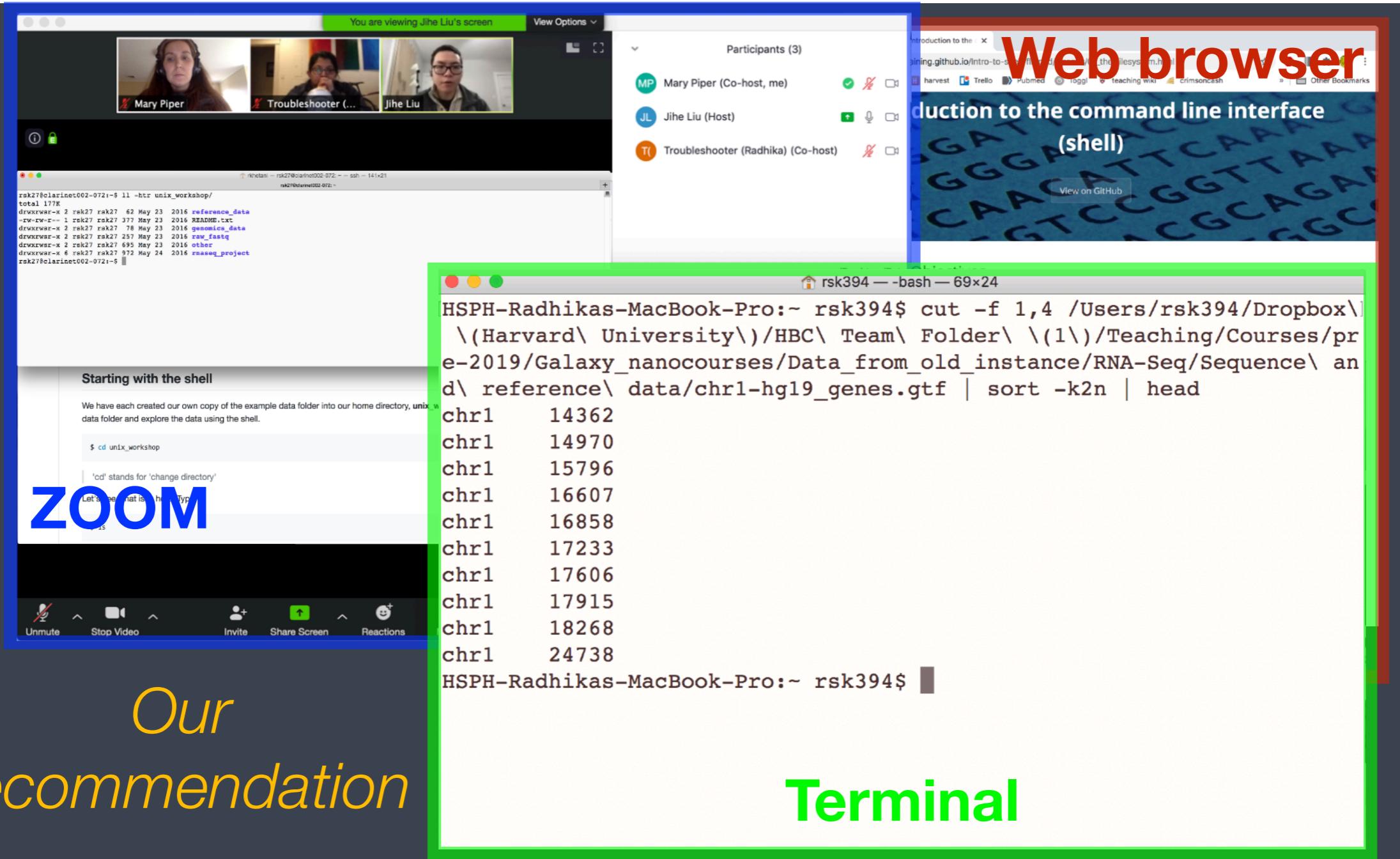
# Single screen & 3 windows?

The image shows a video conference interface with three windows:

- Top Left Window:** A video feed showing three participants: Mary Piper, Troubleshooter (Radhika), and Jihe Liu.
- Top Right Window:** A participant list titled "Participants (3)" showing Mary Piper (Co-host, me), Jihe Liu (Host), and Troubleshooter (Radhika) (Co-host).
- Bottom Window:** A terminal session titled "rsk394 — bash — 69x24" displaying command-line output. The command run was: `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/chrl-hg19_genes.gtf | sort -k2n | head`. The output shows a list of chromosomes and their lengths:chr1 14362  
chr1 14970  
chr1 15796  
chr1 16607  
chr1 16858  
chr1 17233  
chr1 17606  
chr1 17915  
chr1 18268  
chr1 24738

**Bottom Left Text:** "Our recommendation" followed by a large green "Terminal" text.

# Single screen & 3 windows?



# Course participation

- ▶ Please keep your videos on, we would love to see your faces!
- ▶ 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!



# Homework and Expectations

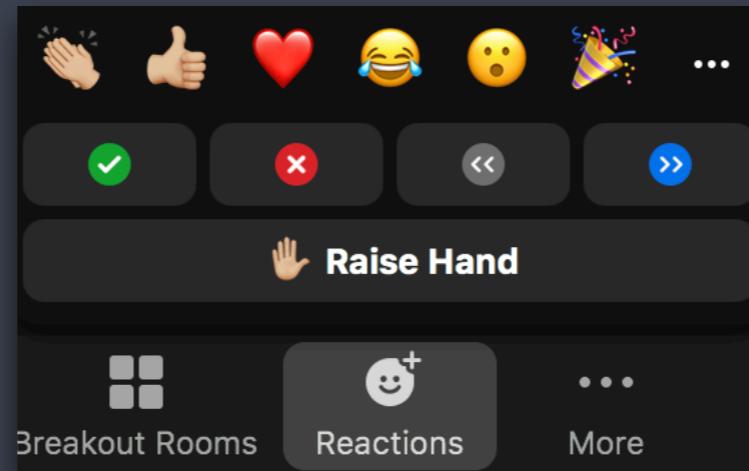
- ❖ At-home lessons and exercises after each session
- ❖ Cover material not previously discussed
- ❖ Provides us feedback to help pace the course appropriately
- ❖ 3-5 hours to complete

# Odds and Ends (1/2)

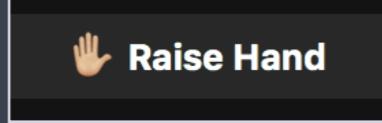
- ❖ Quit/minimize all applications that are not required for class

# Odds and Ends (1/2)

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

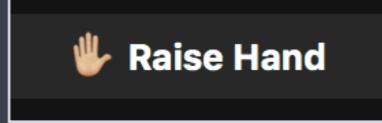


# Odds and Ends (2/2)

- ❖ 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 software?

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

# Thanks!

- Kathleen Keating 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!

*HBC training team:* [hbctraining@hsph.harvard.edu](mailto:hbctraining@hsph.harvard.edu)

*O2 (HMS-RC):* [rchelp@hms.harvard.edu](mailto:rchelp@hms.harvard.edu)

*HBC consulting:* [bioinformatics@hsph.harvard.edu](mailto:bioinformatics@hsph.harvard.edu)

## Twitter

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*HMS-RC:* @hms\_rc