### Day 1 overview

- SSH keys and logging into the AWS
- Basic Bash/Intro to Git
- Vim and Vimtutor
- Review library prep and sequencing (non-DS2)

### Logging into the supercomputer

Open and follow the worksheet SSH\_AWS

(https://github.com/Dowell-Lab/srworkshop/tree/main/day01/worksheets/SSH\_AWS.md)

- You MUST
  - 1. Be logged into your GitHub account
  - 2. Have a terminal application open (see worksheet for details)
- After finishing the worksheet, try to log into the AWS:

```
ssh <github username>@3.20.14.225
```

\*\*replace <github\_username>, including brackets, with your username

If you need help, flag us with a red sticky note If you are successfully logged in, put up a green sticky note

### Basic Bash and Git/GitHub

Open and follow the worksheet Git\_github\_bash

(https://github.com/Dowell-Lab/srworkshop/tree/main/day01/worksheets/Git\_github\_bash.md)

 This worksheet orients you to the class GitHub repository and gets you started with some basic Bash navigation

• Main commands: pwd Print working (current) directory

cd Change directory

ls List contents

If you need help, flag us with a red sticky note If you are done, put up a green sticky note

# Break

If you do not have a working terminal, please log into Google Shell now

(.../srworkshop/day01/worksheets/Google\_shell.md)

### Vim and Vimtutor

- What is Vim?
  - Text editor read, write, and save text files
  - Entirely keyboard-based
  - You CANNOT use your mouse to move the cursor!!!
- Vimtutor is on every Unix/Linux system and teaches you how to use Vim
- Open and follow the worksheet Vimtutor\_crashes\_colors

(.../srworkshop/day01/worksheets/Vimtutor\_and\_Vim\_crashes.md)

If you need help, flag us with a red sticky note If you are done, put up a green sticky note

### Day 1 overview

- SSH keys and logging into the AWS
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- Vim and Vimtutor
- Review library prep and sequencing (non-DS2)

### Homework for day 2

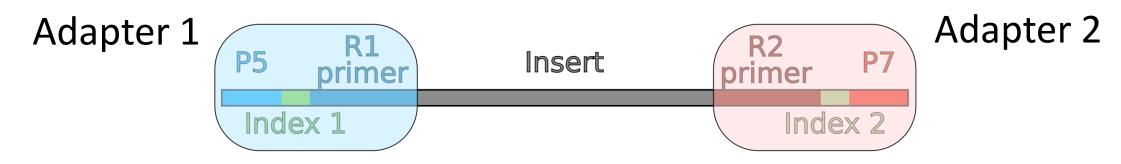
- Videos for day 1 (if not already done)
- Videos for day 2
- Vimtutor
  - Lessons 1 and 2
  - Advanced students should do lessons 3-5
- Day1\_homework (library QC challenge)

### **Short Read Workshop Week 2**

- Day 6: Introduction to R
- Beginning of Day 7: Differential Expression Demo
- Days 7-9: Mini-projects
  - Project A (Room A104) Single Cell RNA-seq
  - Project B (Room A108) Multi-omics: RNA-seq/ChIP-seq(/ATAC-seq)
- End of Day 9: Reconvene and discuss
- Day 10: Potpourri

# Library prep and sequencing

# Anatomy of a library



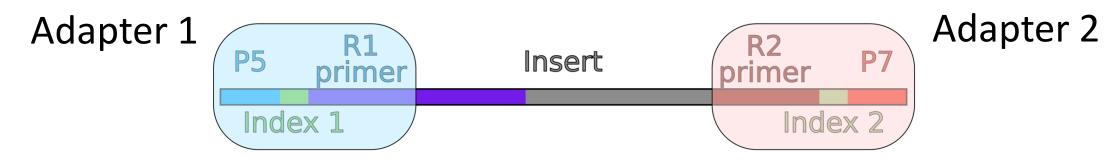
P5/P7 Ends that attach to flow cell

Index 1/2 ID sequences for multiplexing samples

R1/R2 primers Sequencing primers

**Insert** Fragment of sample DNA/cDNA

## Anatomy of a library



P5/P7 Ends that attach to flow cell

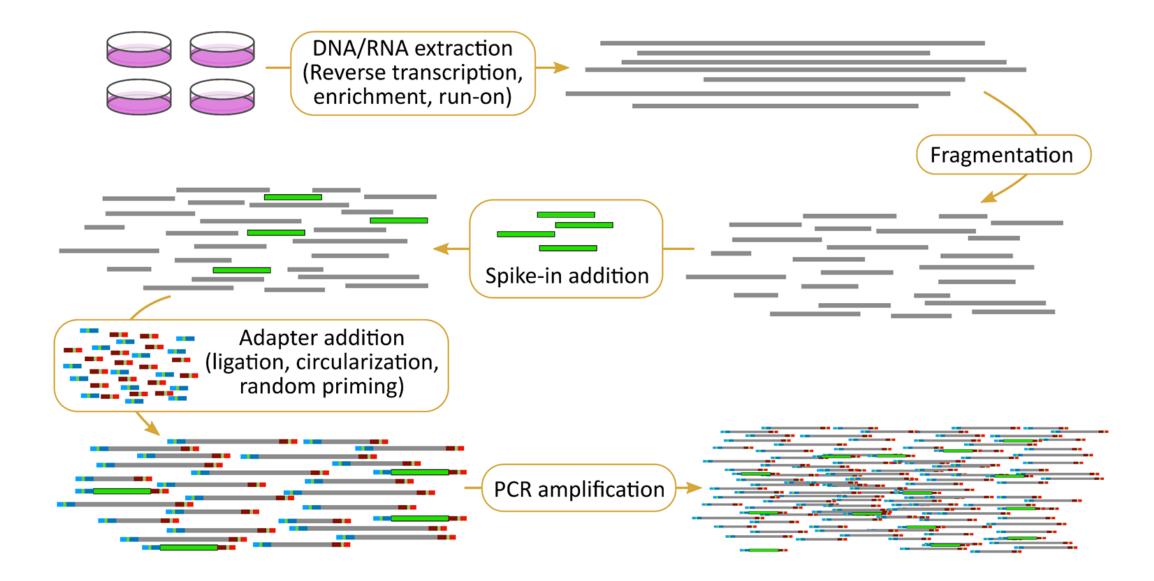
Index 1/2 ID sequences for multiplexing samples

R1/R2 primers Sequencing primers

Insert Fragment of sample DNA/cDNA

Read Sequenced portion of fragment

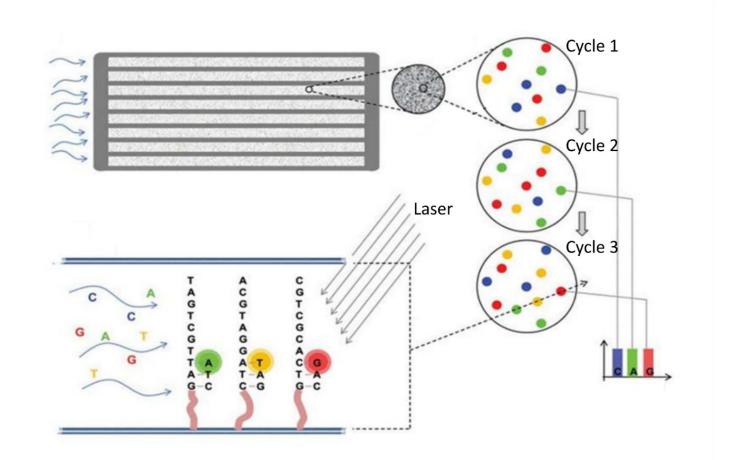
### Creating libraries



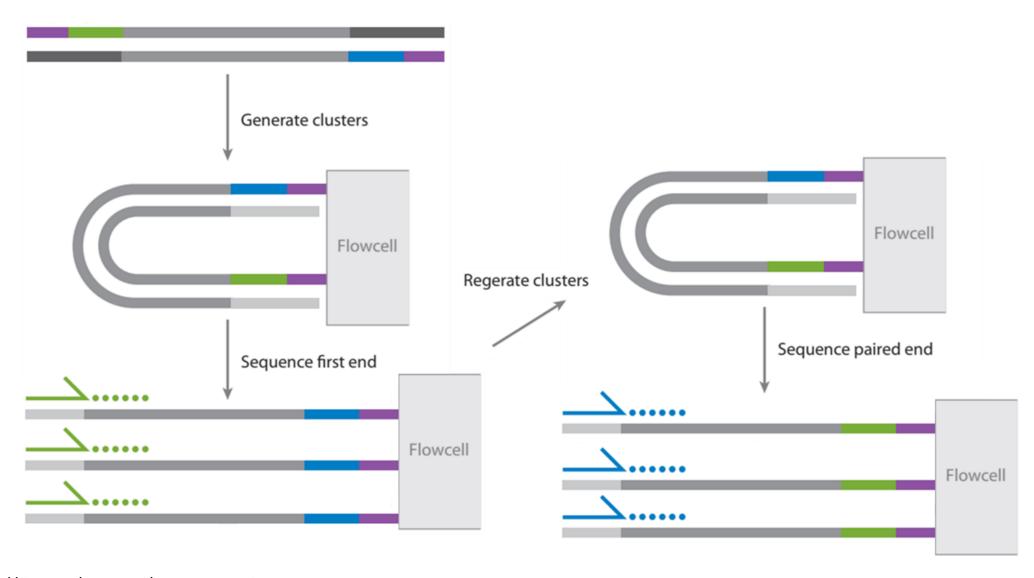
## Illumina sequencing technology

Imaging a slide (flow cell) with millions/billions of DNA clusters by cycling in fluorescent nucleotides

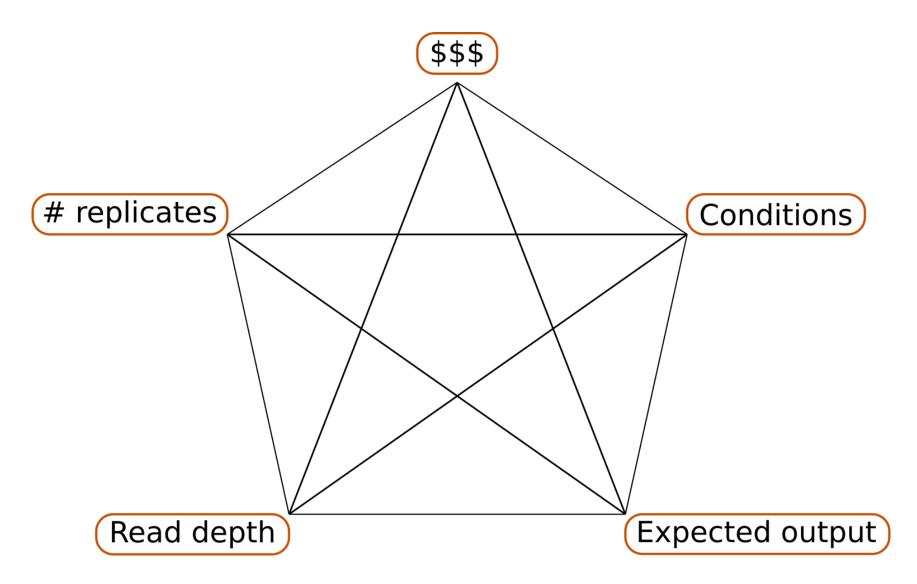
Sequencing:



# Single/paired end sequencing



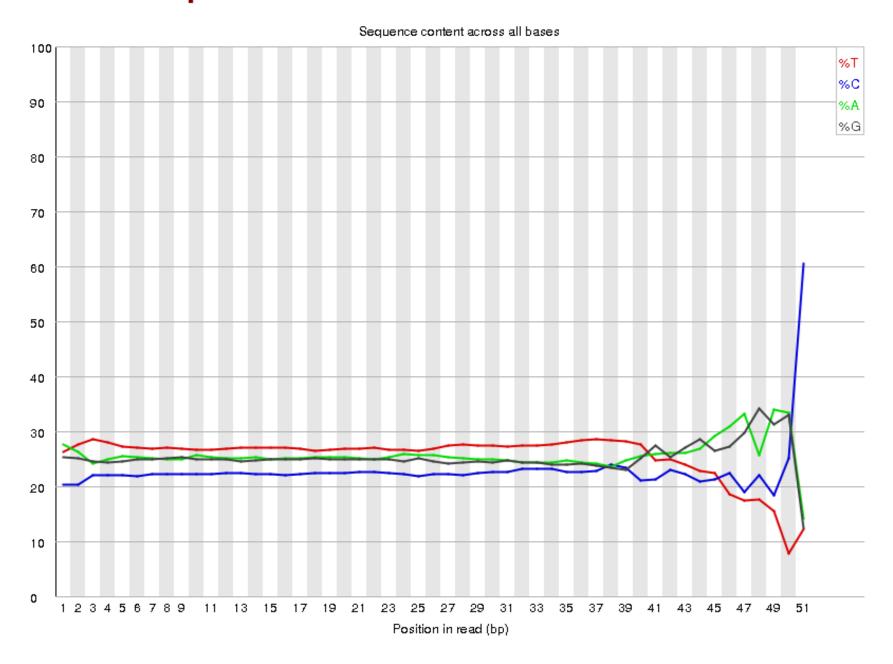
### Designing a sequencing experiment



# Library QC

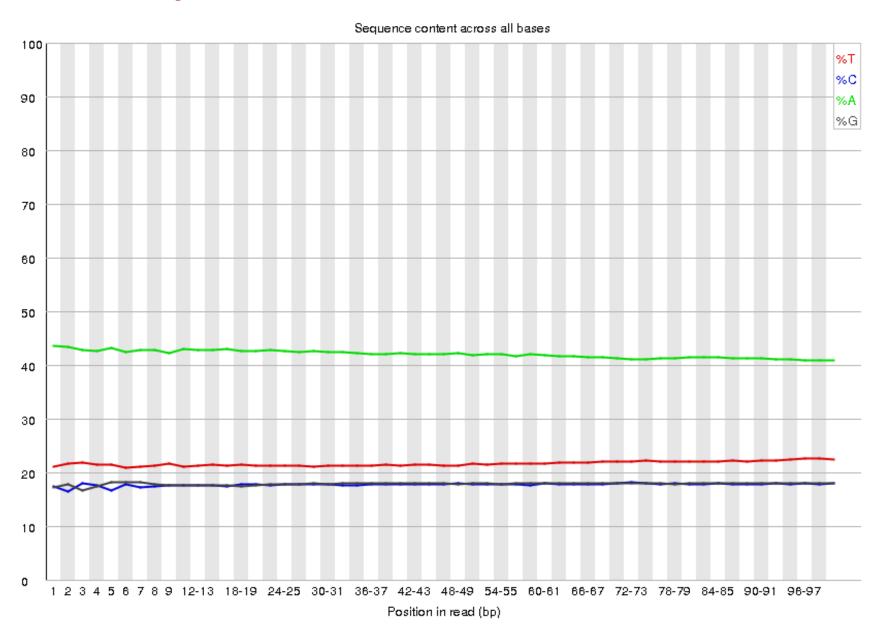
Base diversity
Complexity

#### Per base sequence content



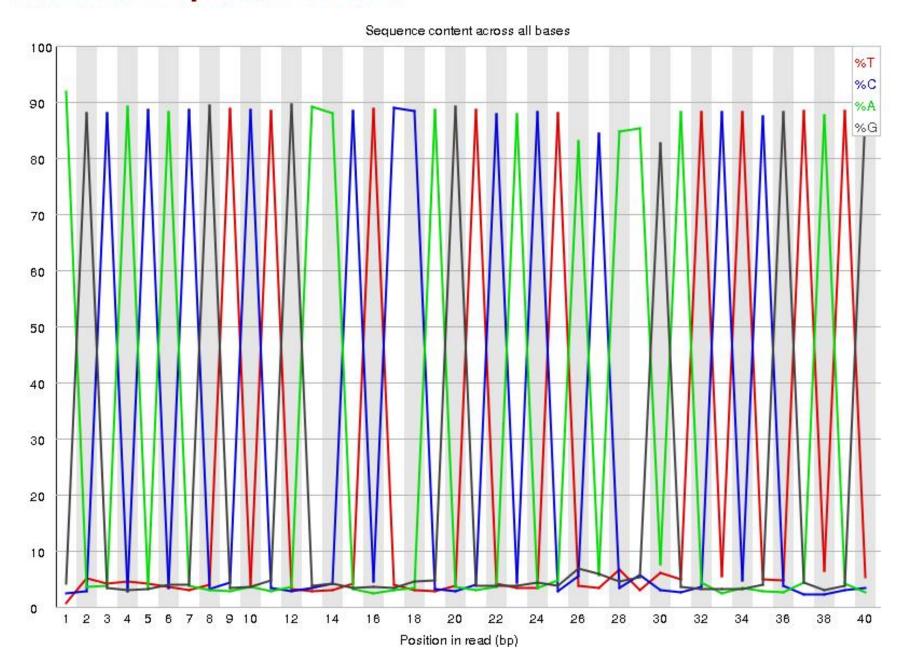
Base diversity
Complexity

#### Per base sequence content



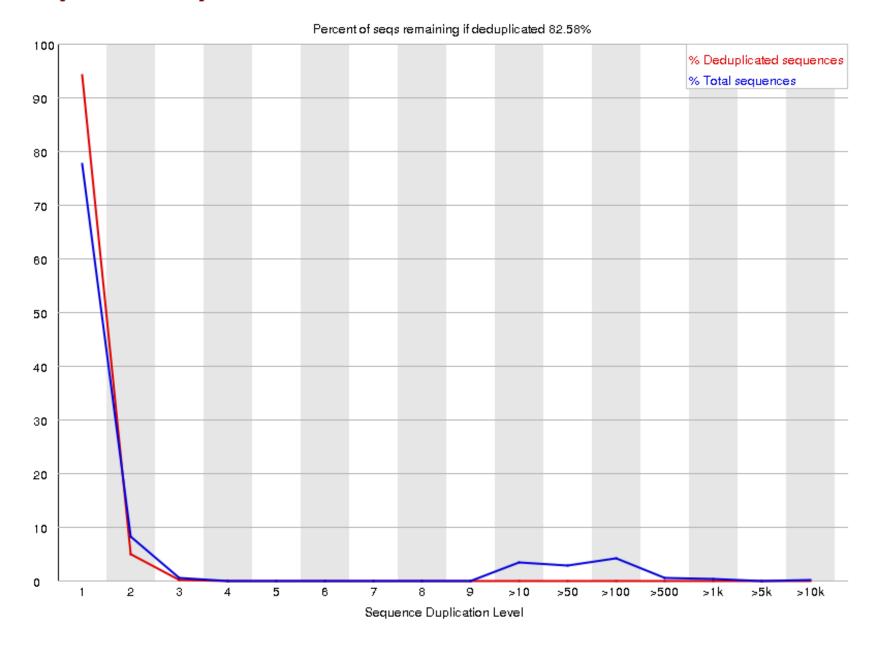
Base diversity
Complexity

#### Per base sequence content



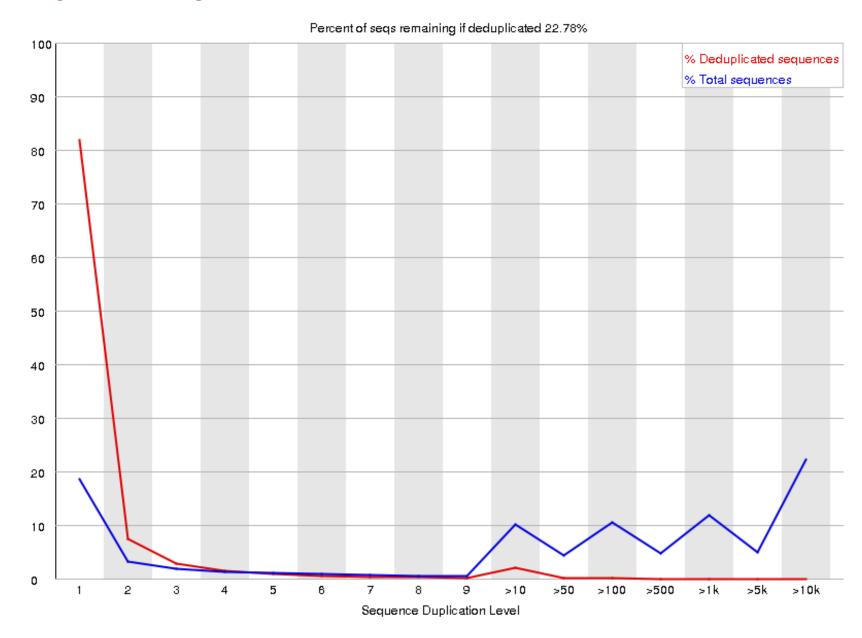
Complexity
Duplication

#### **Sequence Duplication Levels**



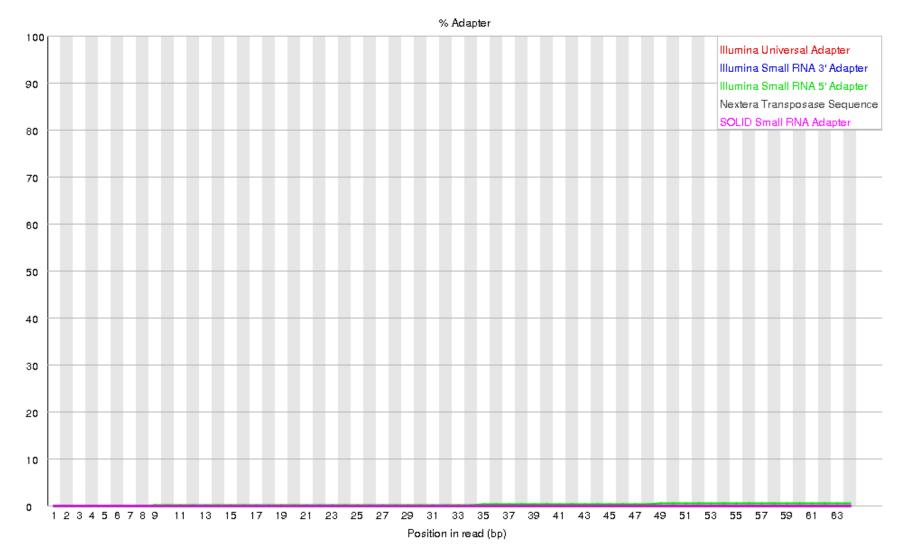
Complexity
Duplication

#### **Sequence Duplication Levels**



#### **Adapter Content**

# Adapter Contamination



#### **Adapter Content**

# Adapter Contamination

