Day 1 overview

- Log on to AWS

- Review library prep and sequencing

- Talk about library QC

- Basic Bash

- Vim and Vimtutor

Logging on to a cluster

Log in

Open terminal on a mac or a bash system on the pc like ubuntu
Type$ hostname
And the computer will tell you your computer's name.

Type$ ssh <username>@<computername>

The first time you log in it will ask you:
Are you sure you want to continue. Type$ yes

Super computers will either use a ssh key or will ask you for a password. If you type a password, you will see nothing. That's normal! It's a feature not a bug.
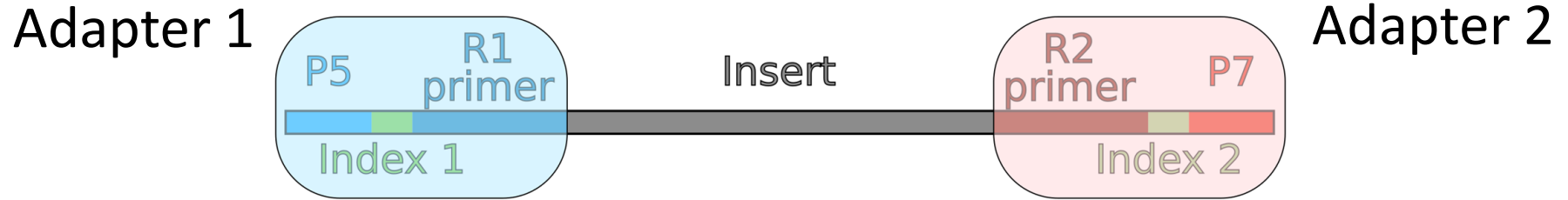
To confirm you are on the super computer
Type$ hostname
And the computer will tell you the super computer's name.

Log out

Type$ logout

Library prep and sequencing

Anatomy of a library

P5/P7 — Ends that attach to flow cell

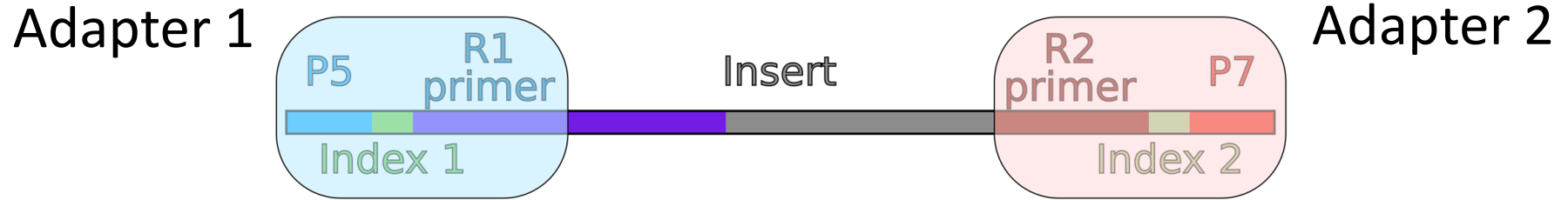Index 1/2 — ID sequences for multiplexing samples

R1/R2 primers — Sequencing primers

Insert — Fragment of sample DNA/cDNA

Anatomy of a library

Adapter 1

P5

R1 primer

Index 1

Insert

R2 primer

P7

Index 2

Adapter 2

P5/P7 — Ends that attach to flow cell

Index 1/2 — ID sequences for multiplexing samples
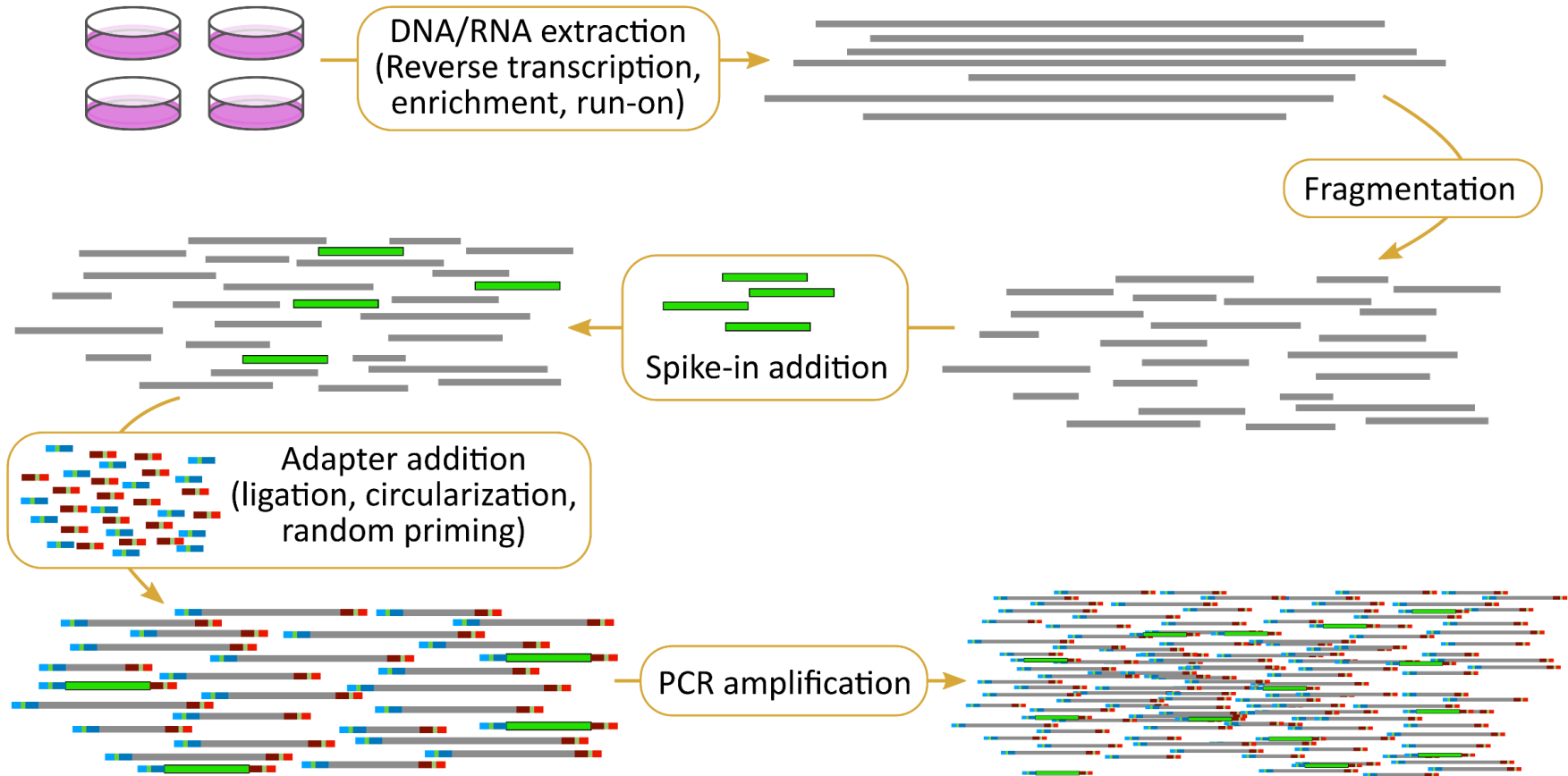
R1/R2 primers — Sequencing primers

Insert — Fragment of sample DNA/cDNA
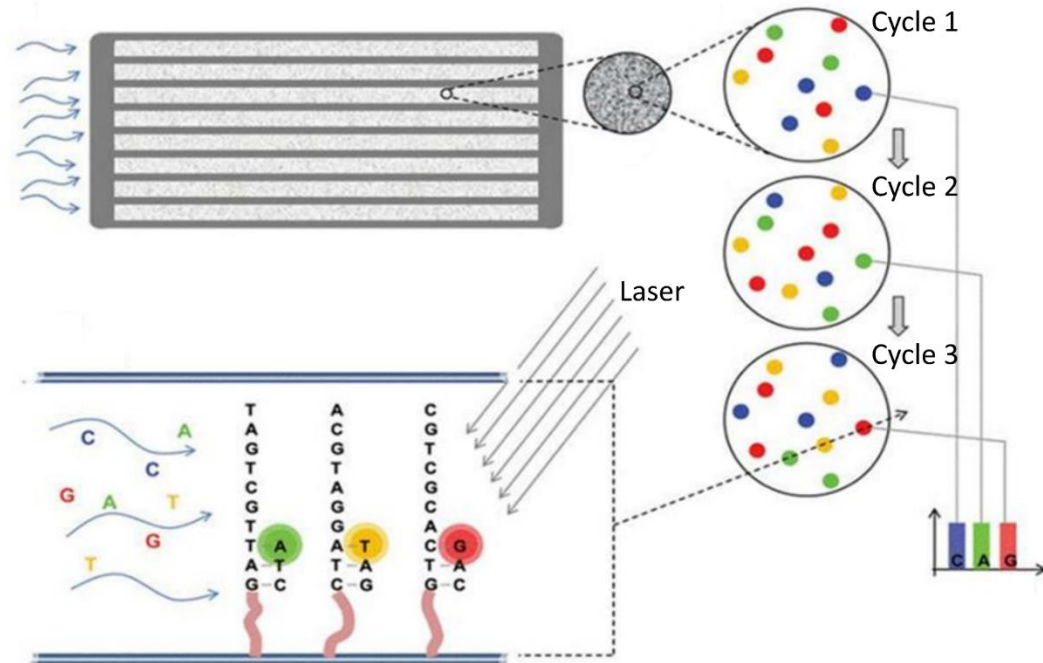
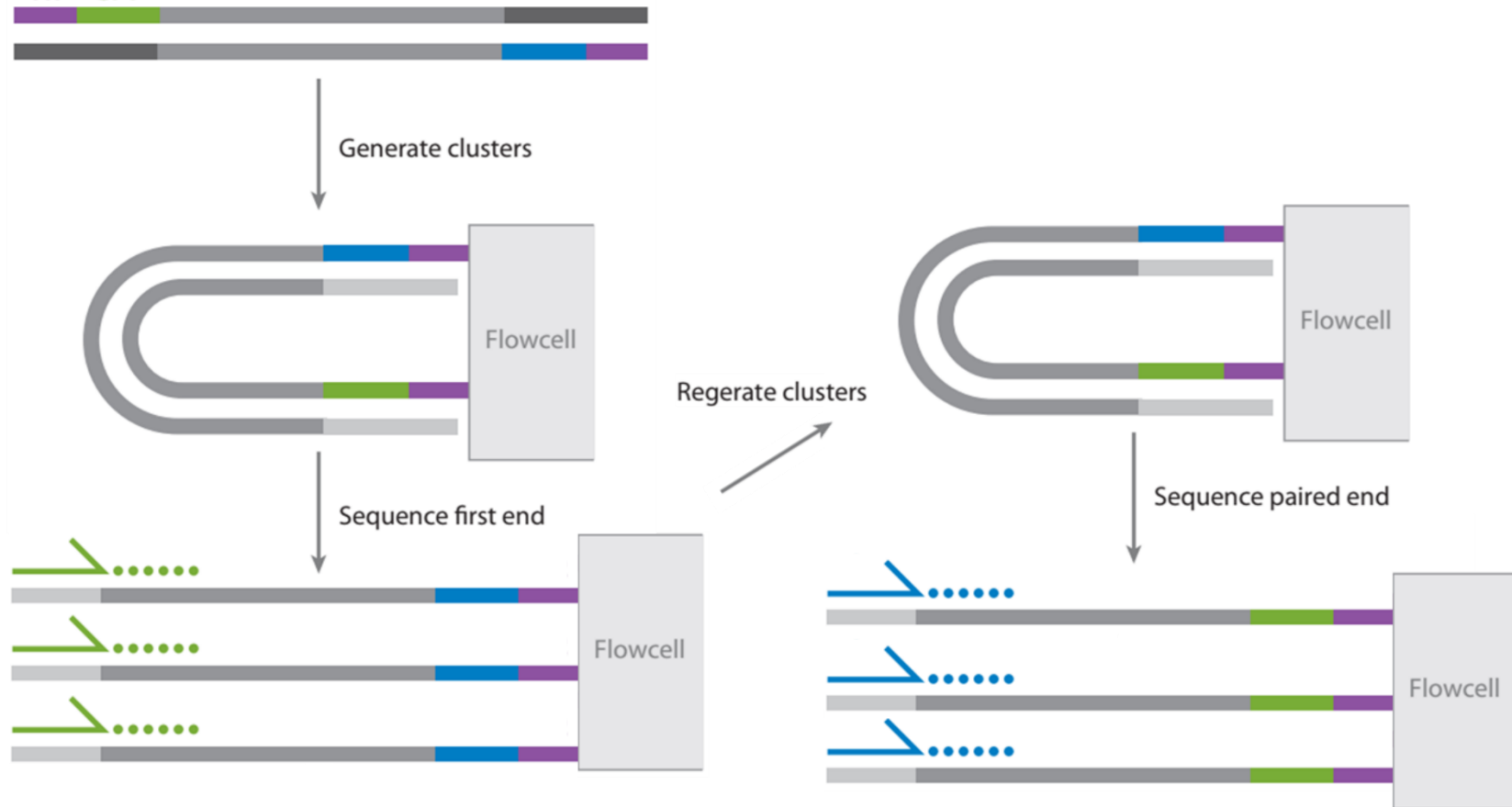Read — Sequenced portion of fragment

Creating libraries

Illumina sequencing technology

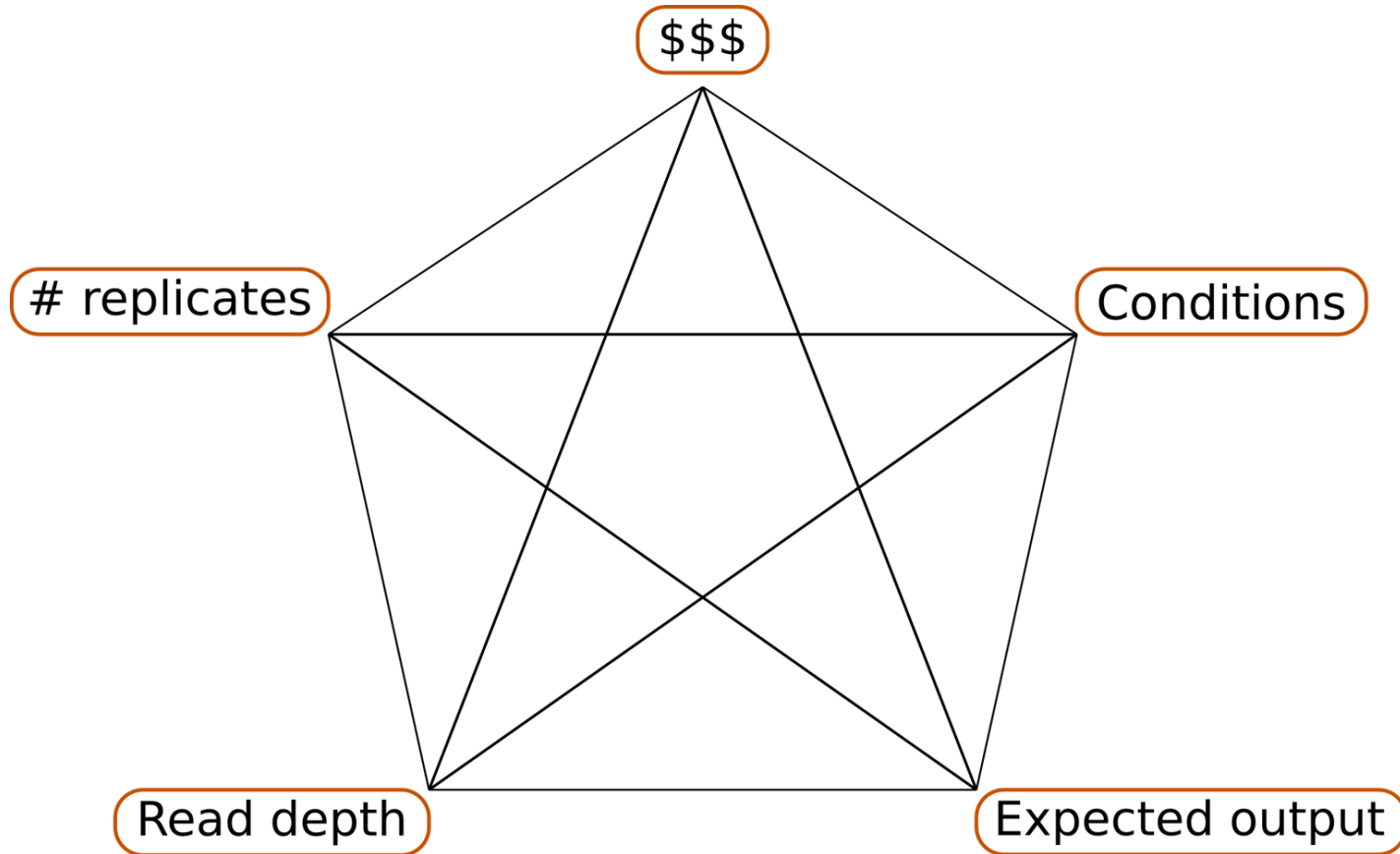Imaging a slide (flow cell) with millions/billions of DNA clusters by cycling in fluorescent nucleotides

Sequencing:

Single/paired end sequencing
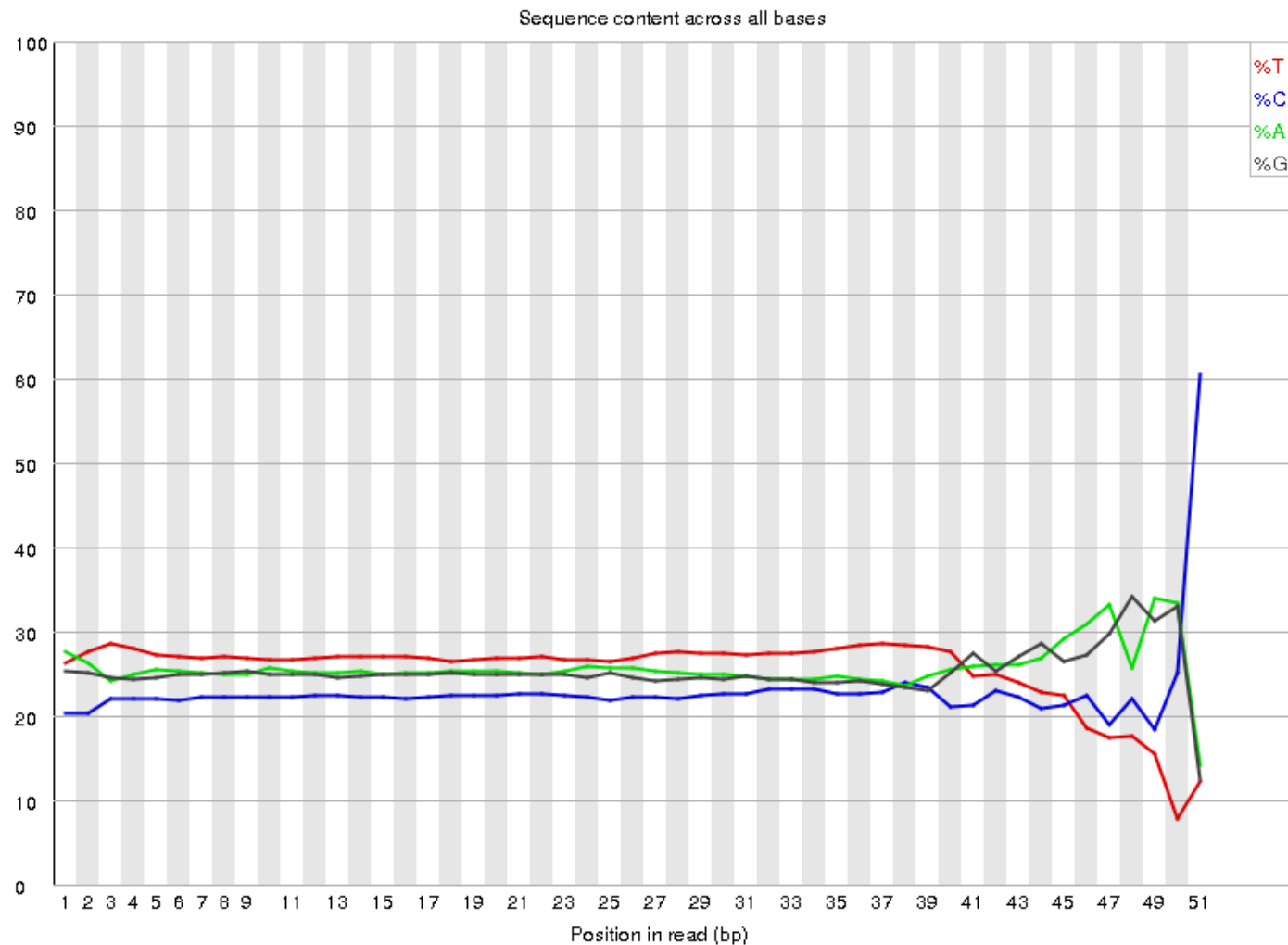
Designing a sequencing experiment

Library QC

FastQC

Base diversity
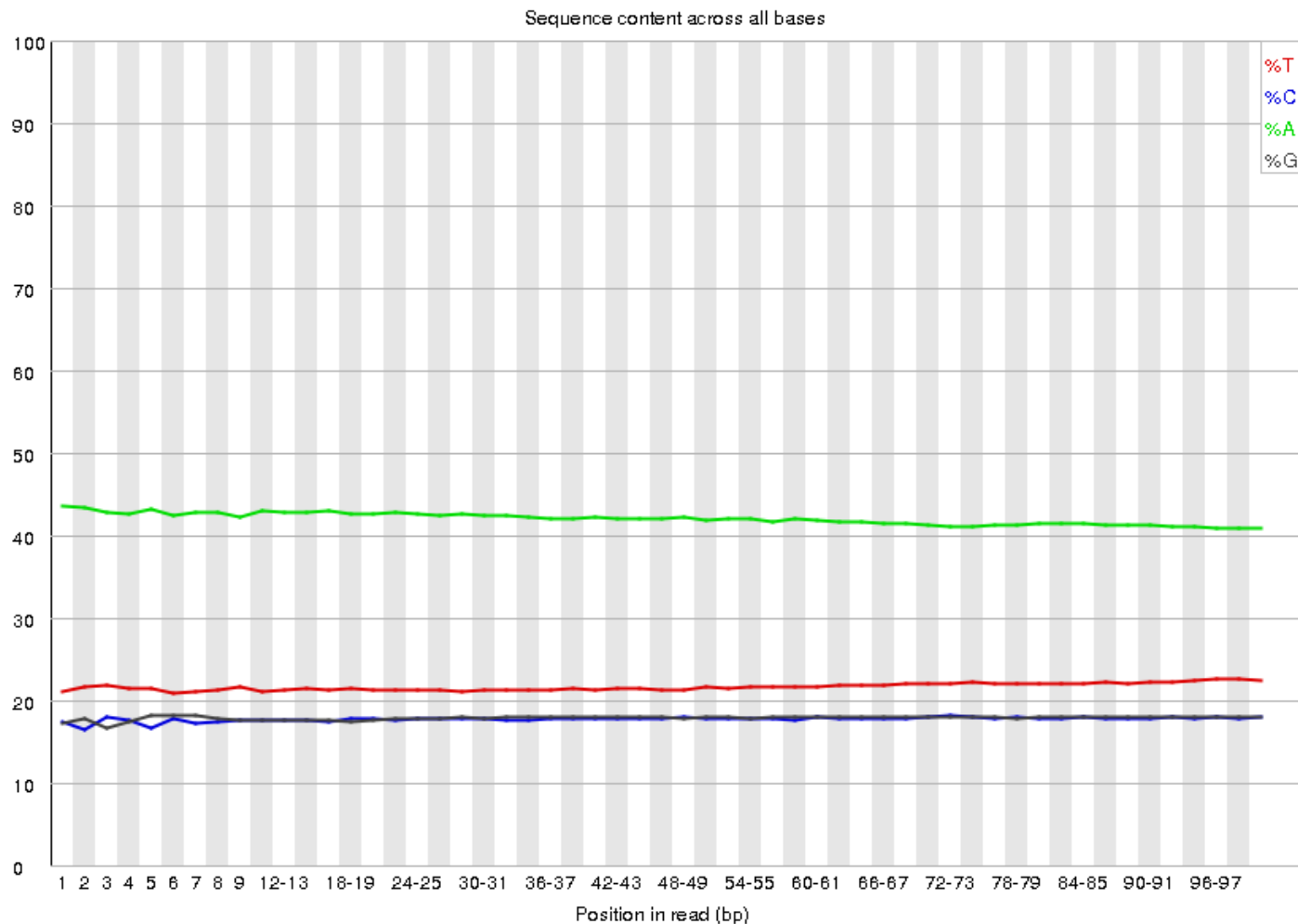
Complexity

Per base sequence content
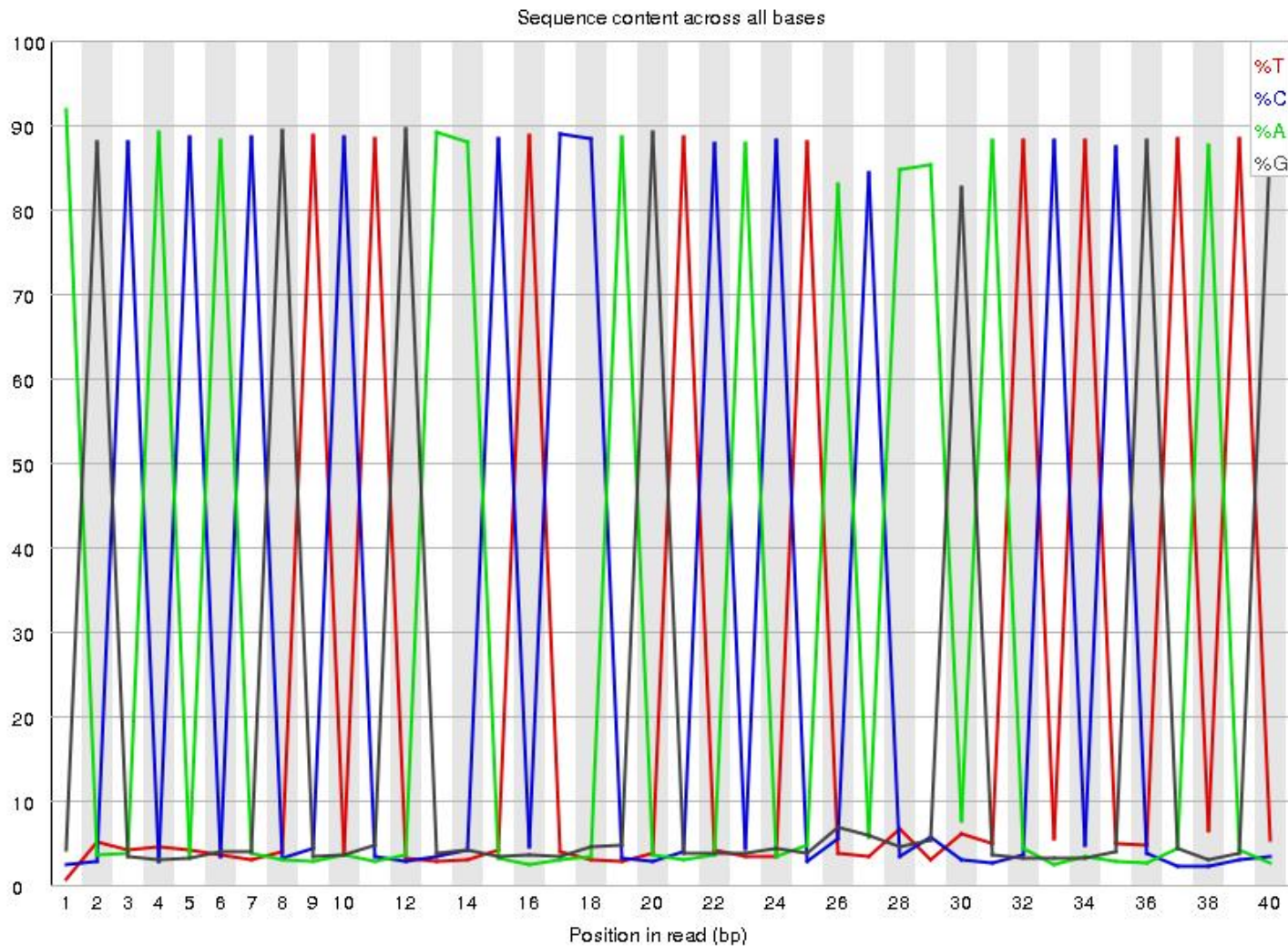
FastQC
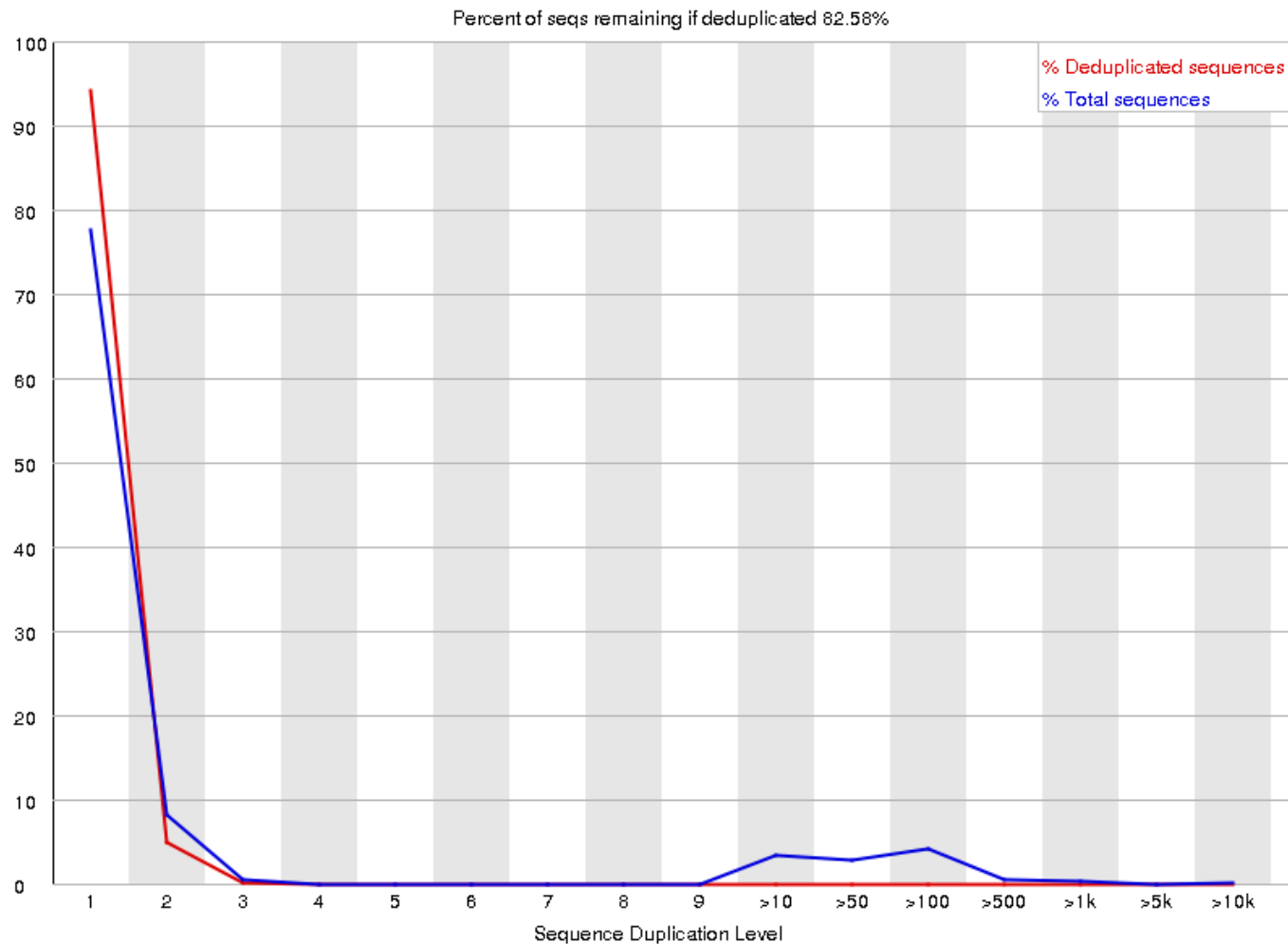
Base diversity

Complexity

FastQC

Base diversity

Complexity

FastQC

Complexity

Duplication

Sequence Duplication Levels

Percent of seqs remaining if deduplicated 82.58%

% Deduplicated sequences
% Total sequences

Sequence Duplication Level

FastQC

Complexity

Duplication

Sequence Duplication Levels

Percent of seqs remaining if deduplicated 22.78%

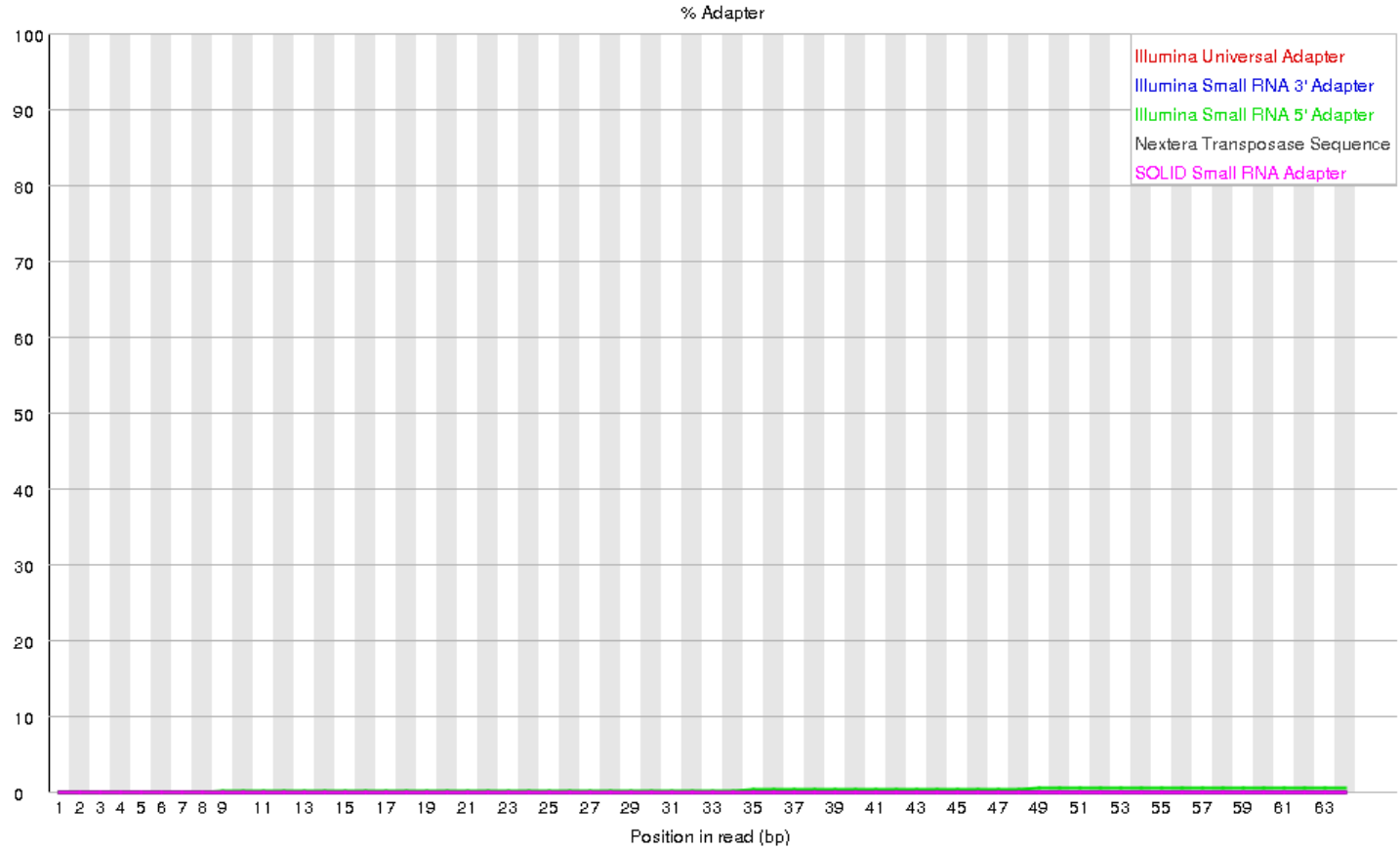% Deduplicated sequences
% Total sequences
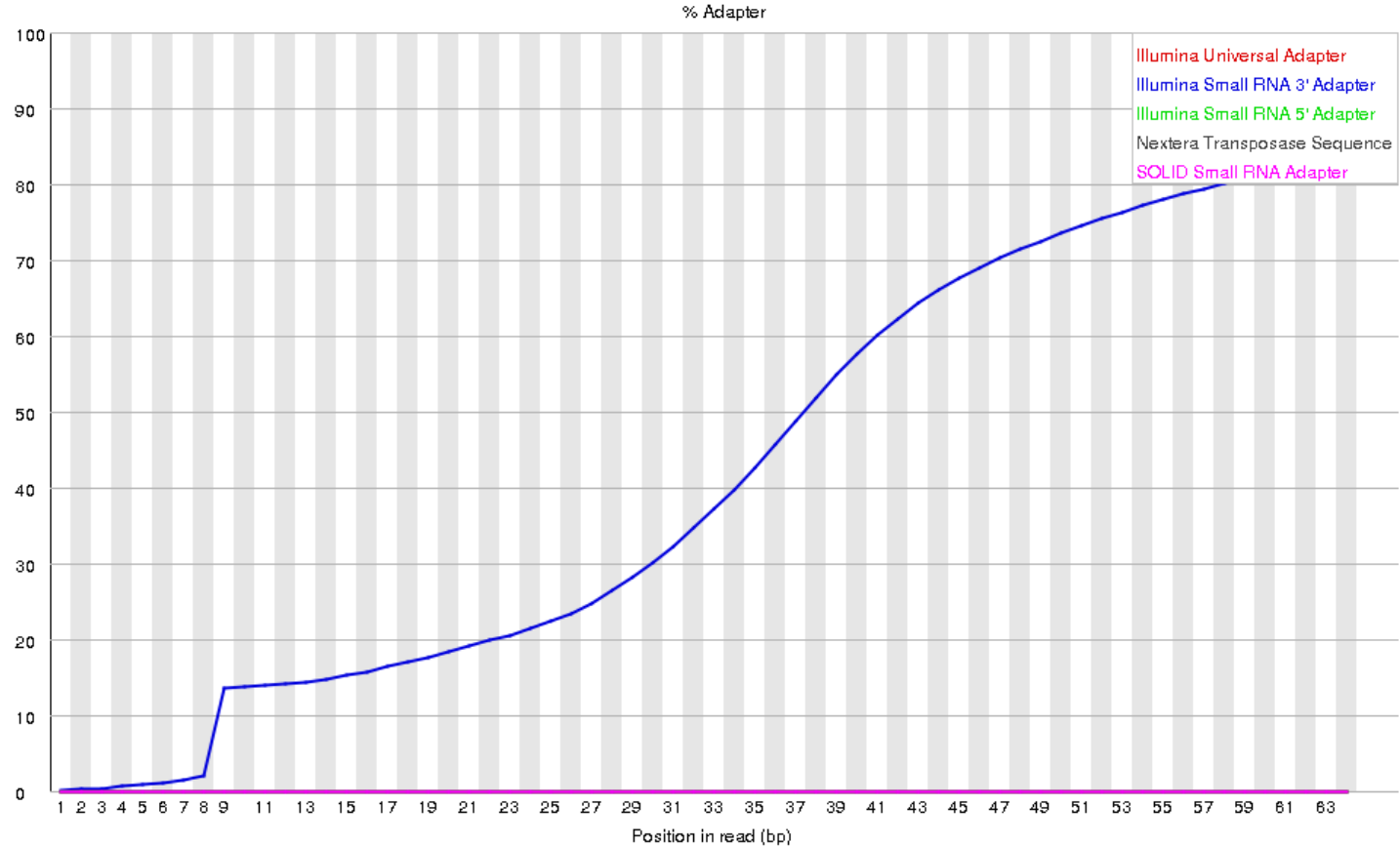
Sequence Duplication Level
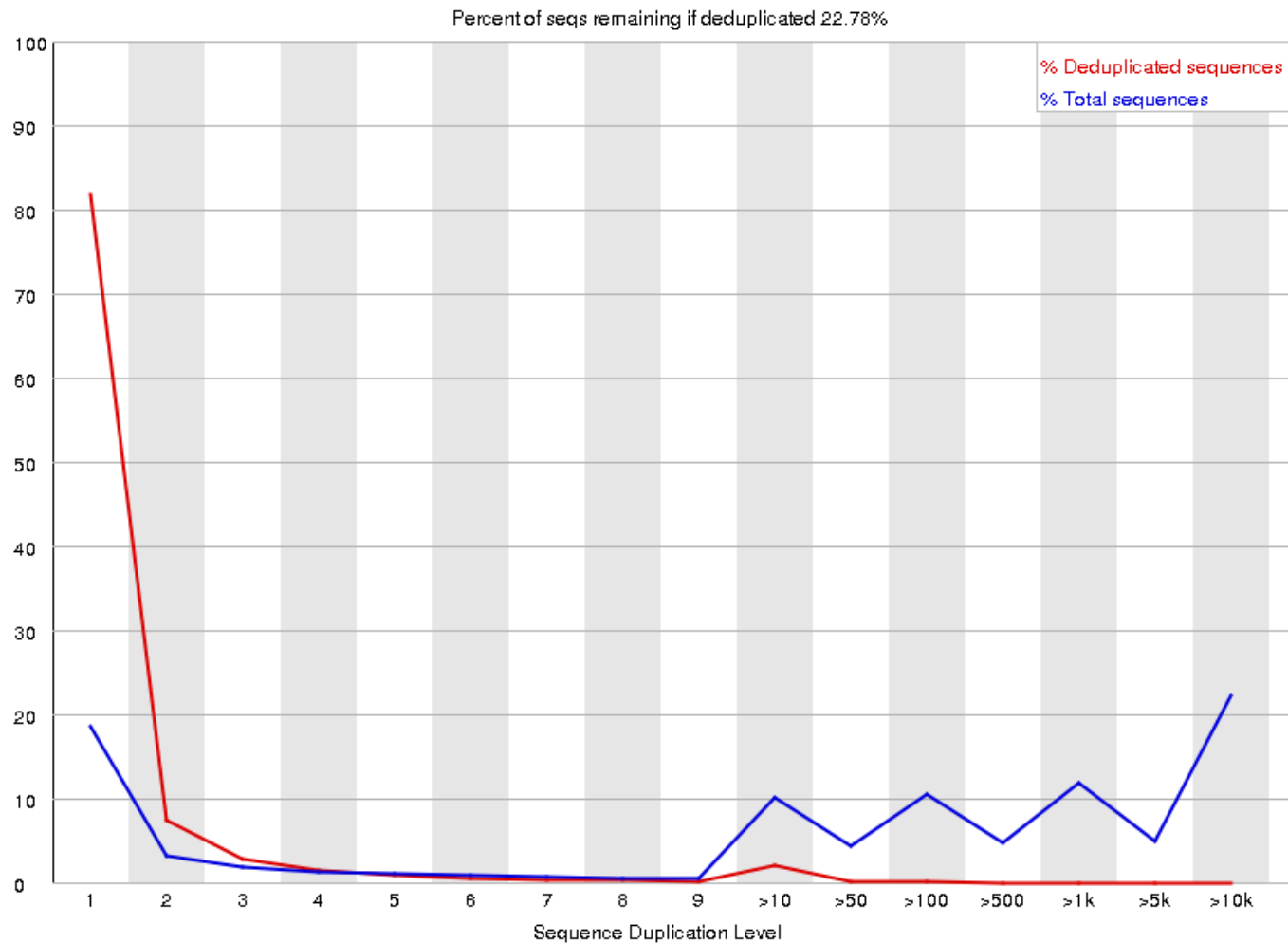
FastQC

Adapter Contamination

FastQC

Adapter
Contamination

Adapter Content

Break

(If you haven't gotten onto the AWS, please log into Google Shell now)

Basic Bash

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 linux system and teaches you how to use vim – open it now

```
~$ vimtutor
```

Variables in Bash (see worksheet)