NextGen sequencing and high-performance computing

QB Bootcamp, Day 2
Wednesday, 6 September 2023
1:00pm - 1:30pm

The Stillman uses high-throughput DNA sequencing to study the dynamics of DNA replication initiation and progression

Domain within the helicase subunit Mcm4 integrates multiple kinase signals to control DNA replication initiation and fork progression

Yi-Jun Sheu[a], Justin B. Kinney[a], Armelle Lengronne[b], Philippe Pasero[b], and Bruce Stillman[a,1]

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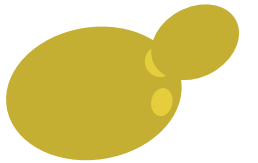
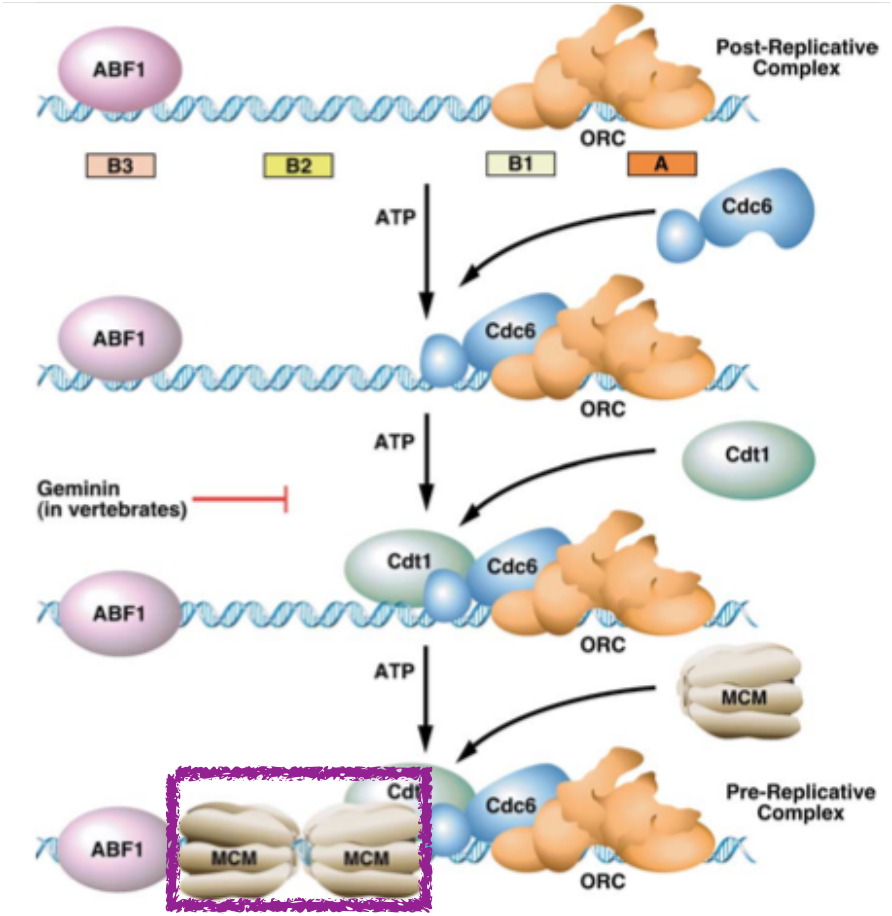
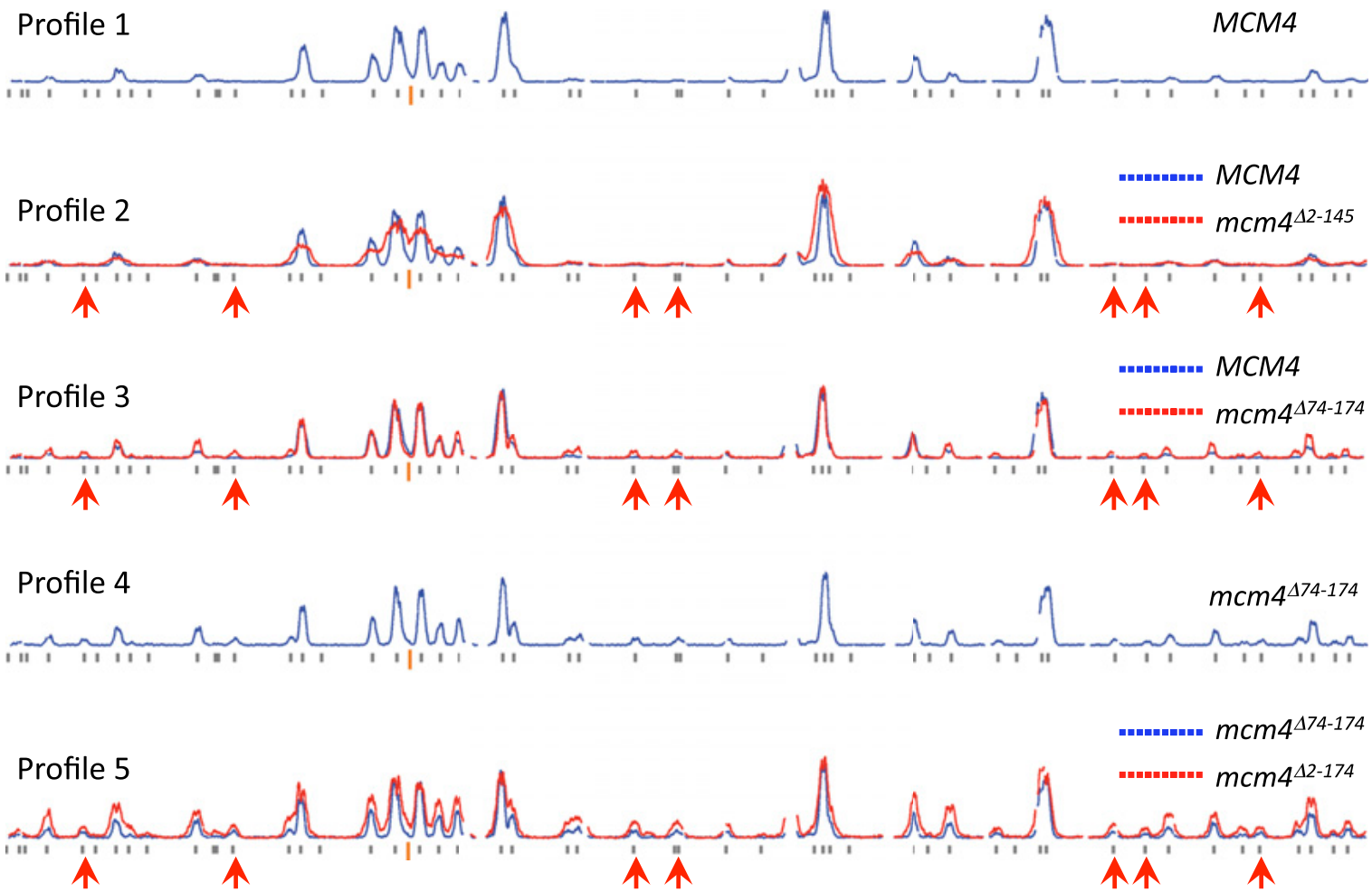


S. cerevisiae



Here are some examples of the published replication profiles



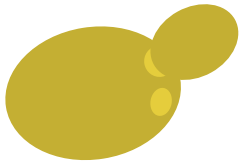
Sheu et al., 2014, PNAS

~300 separate loci direct DNA replication initiation in *Saccharomyces cerevisiae*

ARS: autonomously replicating sequence

old ssDNA

new ssDNA



S. cerevisiae

G1 phase

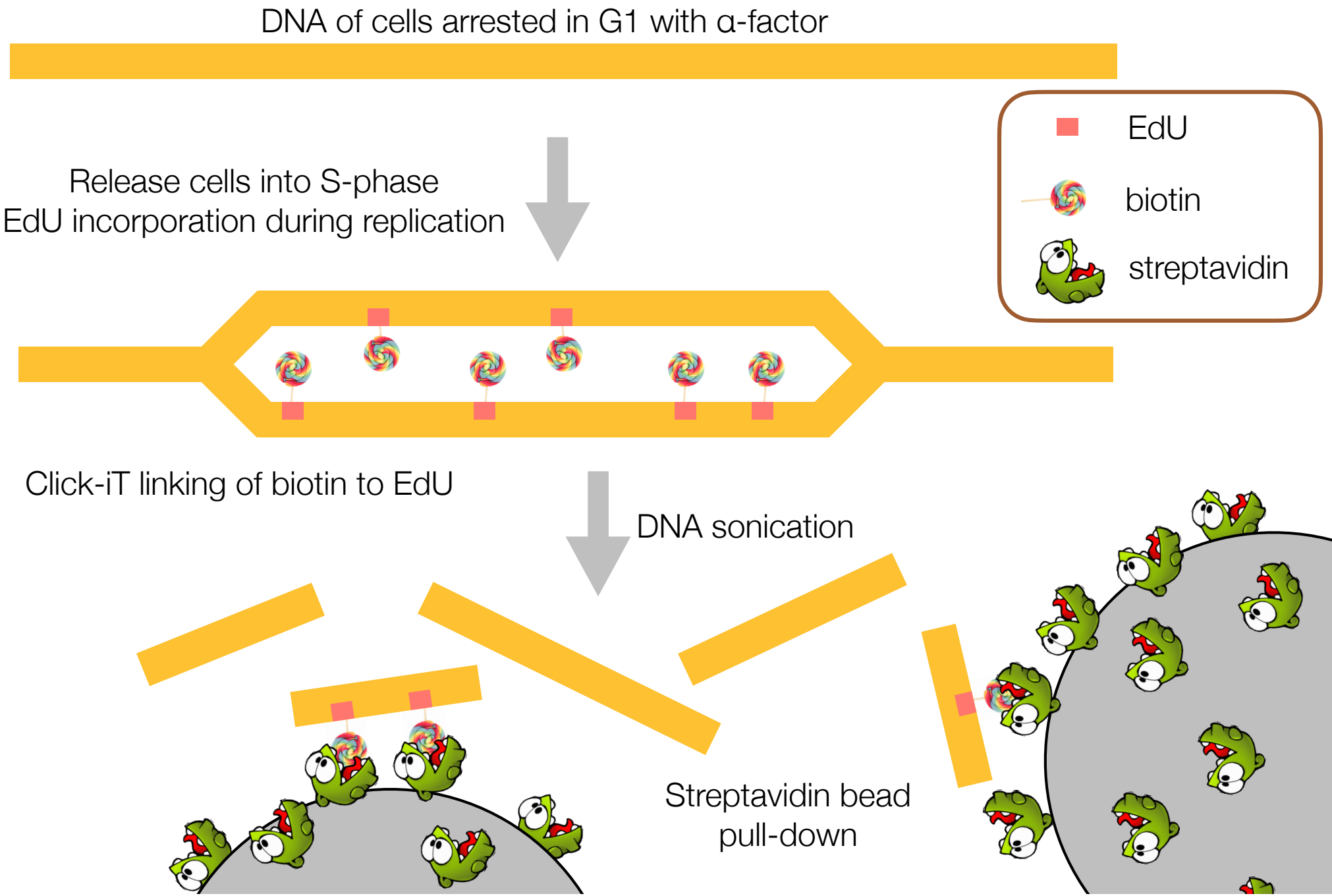
pre-RC

S phase

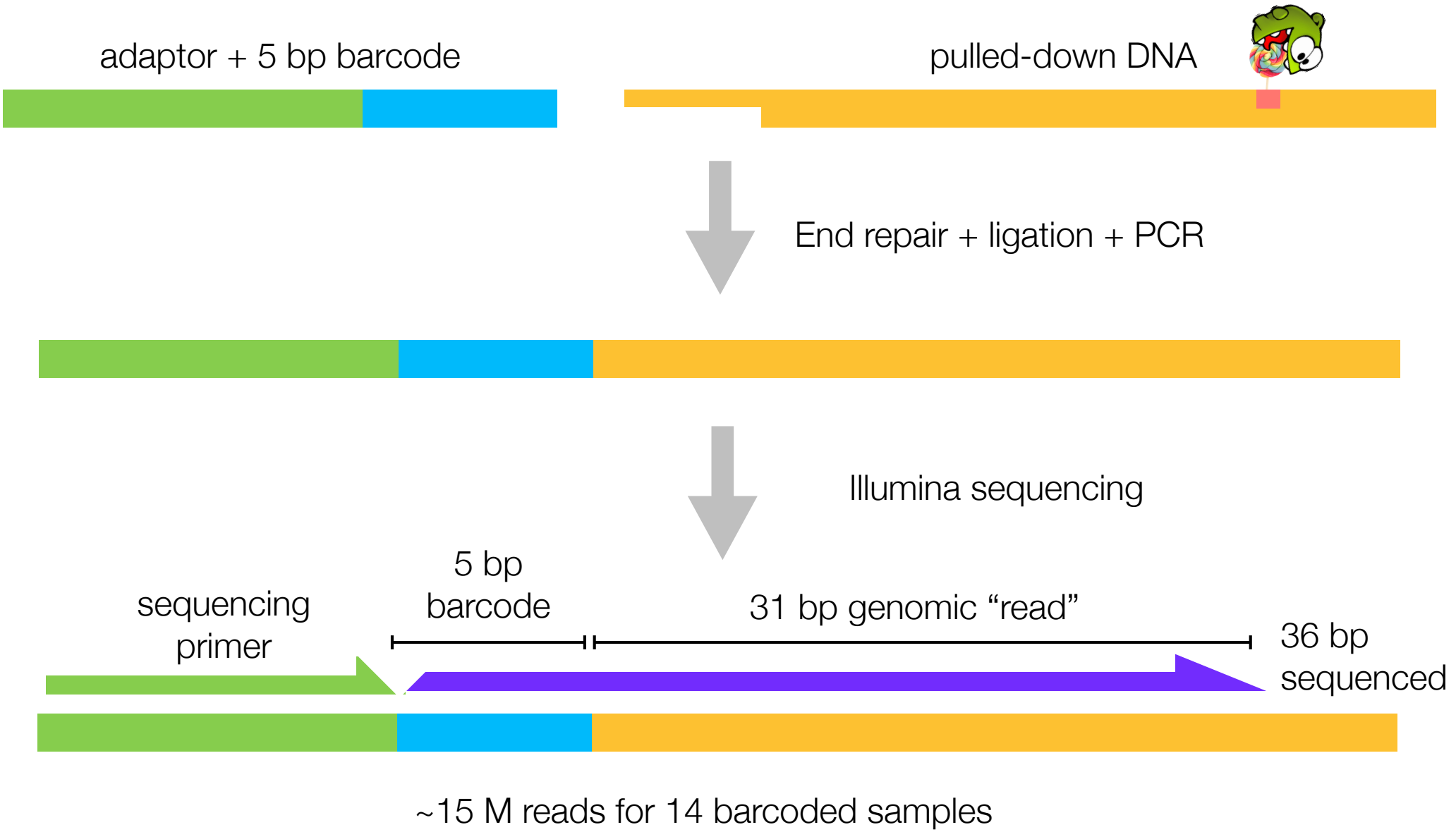
Fork

Fork

Newly replicated DNA can be isolated using an EdU pull-down assay



Sequencing of pulled-down DNA allows replication to be mapped genome-wide

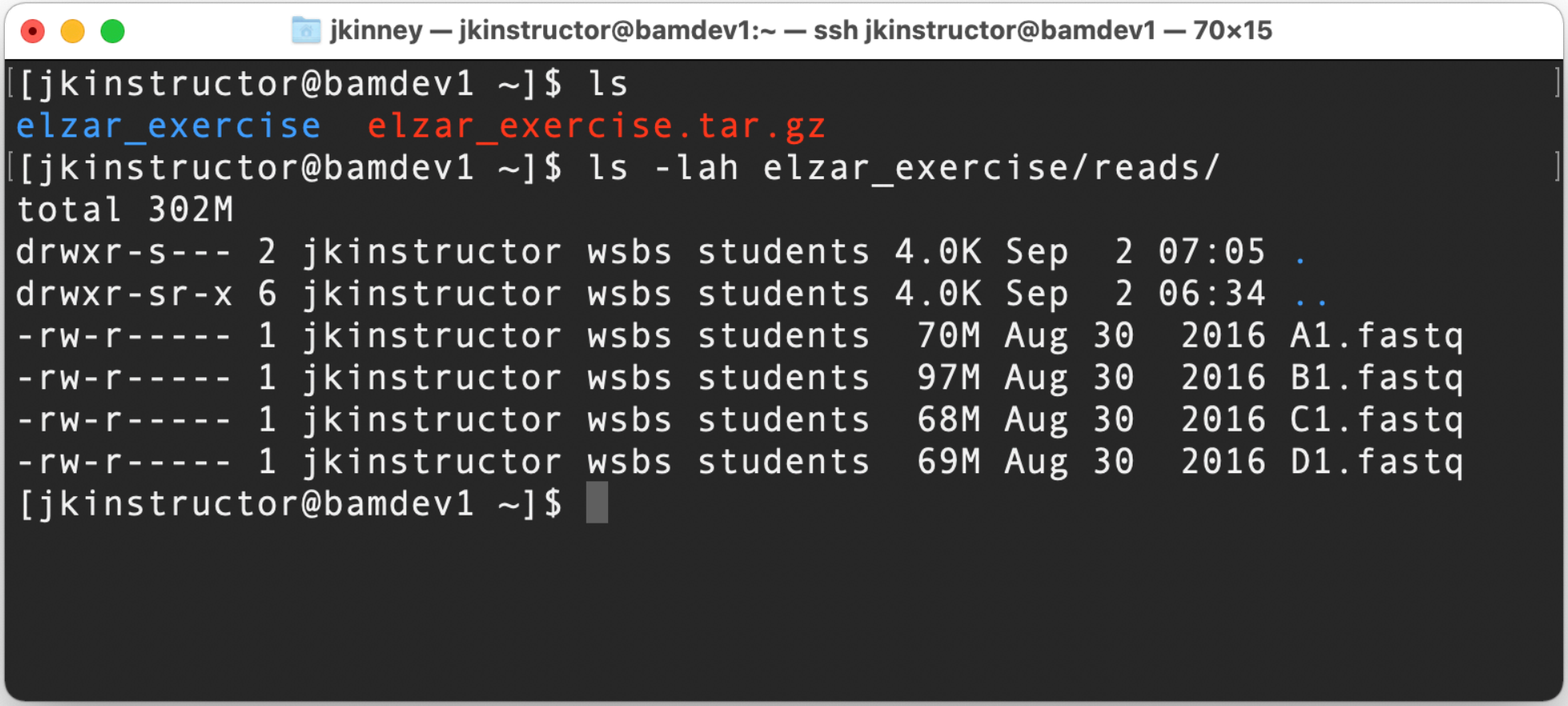


~15 M reads for 14 barcoded samples

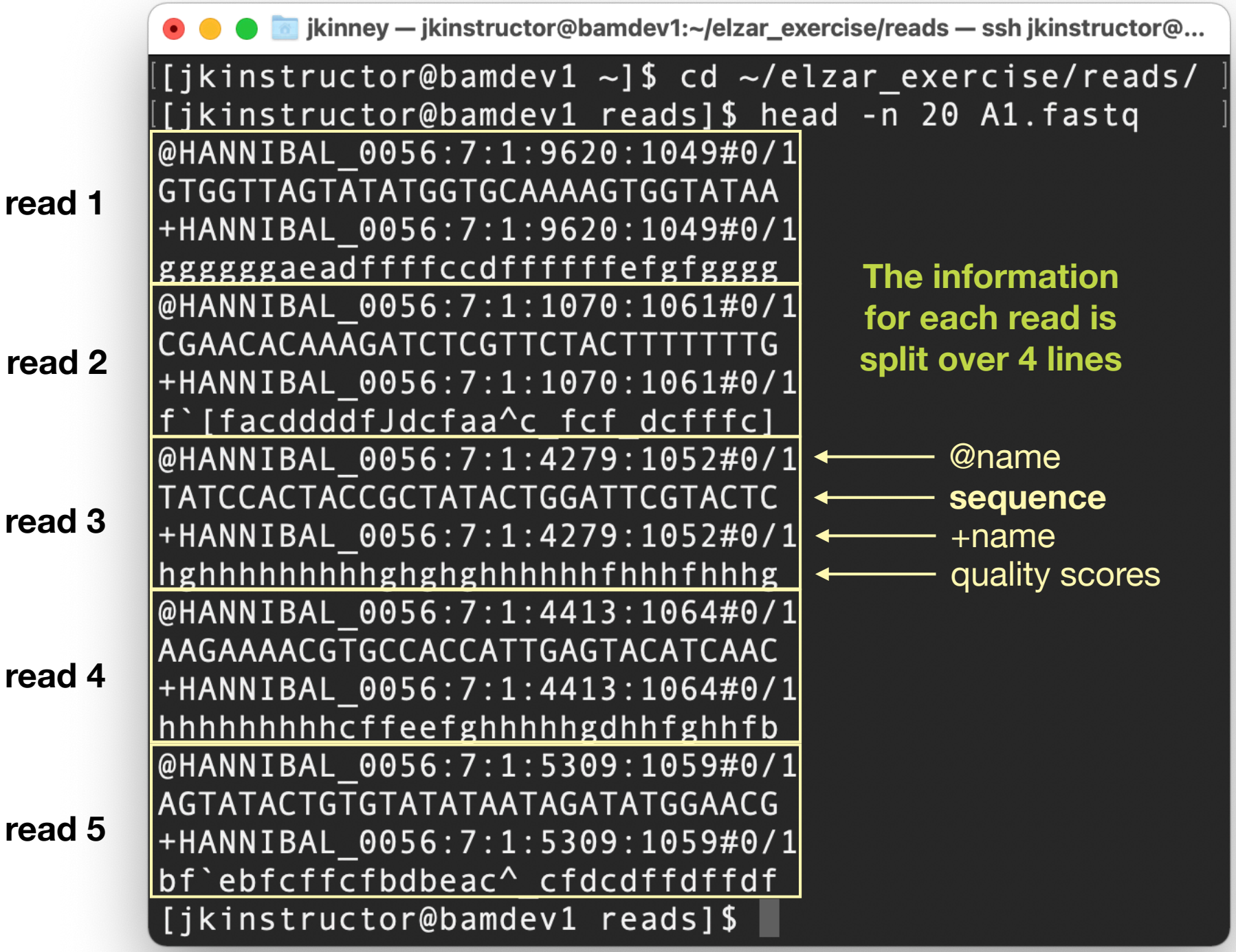
We will analyze FASTQ read files from 4 different samples

There are four FASTQ files in the reads/ directory

Each FASTQ files is ~60-100 megabytes



This is what a FASTQ file looks like (circa 2009)



The yeast genome is in FASTA format

```
[[jkinstructor@bamdev1 ~]$ cd elzar_exercise/genome/
[[jkinstructor@bamdev1 genome]$ head genome.fasta
>1 ref|NC_001133| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=I] [note=R64-1-1]
CCACACCACACCCACACACCCACACACCACACCACACACCACACCACACCCACACACACA
CATCCTAACACTACCCTAACACAGCCCTAATCTAACCCTGGCCAACCTGTCTCTCAACTT
ACCCTCCATTACCCTGCCTCCACTCGTTACCCTGTCCCATTCAACCATACCACTCCGAAC
CACCATCCATCCCTCTACTTACTACCACTCACCCACCGTTACCCTCCAATTACCCATATC
CAACCCACTGCCACTTACCCTACCATTACCCTACCATCCACCATGACCTACTCACCATAC
TGTTCTTCTACCCACCATATTGAAACGCTAACAAATGATCGTAAATAACACACACGTGCT
TACCCTACCACTTTATACCACCACCACATGCCATACTCACCCTCACTTGTATACTGATTT
TACGTACGCACACGGATGCTACAGTATATACCATCTCAAACTTACCCTACTCTCAGATTC
CACTTCACTCCATGGCCCATCTCTCACTGAATCAGTACCAAATGCACTCACATCATTATG
[jkinstructor@bamdev1 genome]$
```

Each header line starts with '>'

The corresponding sequence follows, usually split over lines 80bp long

genome.fasta contains sequences #1 to #16, representing the 16 chromosomes

```
[jkinstructor@bamdev1 genome]$ cat genome.fasta | grep '>'
>1 ref|NC_001133| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=I] [note=R64-1-1]
>2 ref|NC_001134| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=II] [note=R64-1-1]
>3 ref|NC_001135| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=III] [note=R64-1-1]
>4 ref|NC_001136| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=IV] [note=R64-1-1]
>5 ref|NC_001137| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=V] [note=R64-1-1]
>6 ref|NC_001138| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=VI] [note=R64-1-1]
>7 ref|NC_001139| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=VII] [note=R64-1-1]
>8 ref|NC_001140| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=VIII] [note=R64-1-1]
>9 ref|NC_001141| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=IX] [note=R64-1-1]
>10 ref|NC_001142| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=X] [note=R64-1-1]
>11 ref|NC_001143| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=XI] [note=R64-1-1]
>12 ref|NC_001144| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=XII] [note=R64-1-1]
>13 ref|NC_001145| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=XIII] [note=R64-1-1]
>14 ref|NC_001146| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=XIV] [note=R64-1-1]
>15 ref|NC_001147| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=XV] [note=R64-1-1]
>16 ref|NC_001148| [org=Saccharomyces cerevisiae] [strain=S288C] [moltype=genomic] [chromosome=XVI] [note=R64-1-1]
[jkinstructor@bamdev1 genome]$
```

We will map reads to the genome on the cluster, then analyze the resulting .bed files on our local machines

A1.fastq + genome.fasta

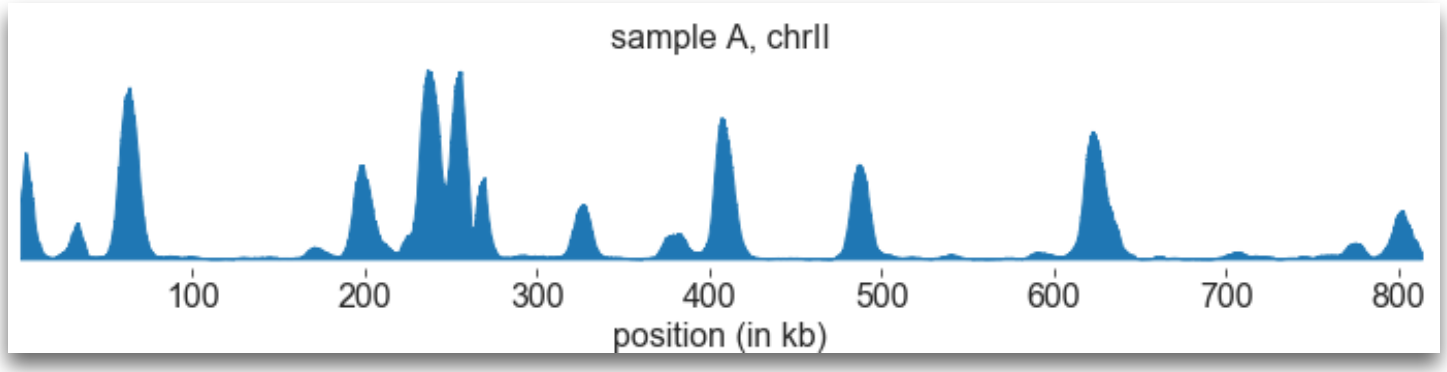
cluster analysis
(bwa + samtools + bedtools)

A1.pileup.bed

```
browser position chrIV:1-1531933
track type=bedGraph visibility=2 name="A1" description="A1"
chrI    1       31      2
chrI    32      62      0
chrI    63      93      1
chrI    94      124     0
chrI    125     155     3
chrI    156     186     0
chrI    187     217     0
chrI    218     248     0
```

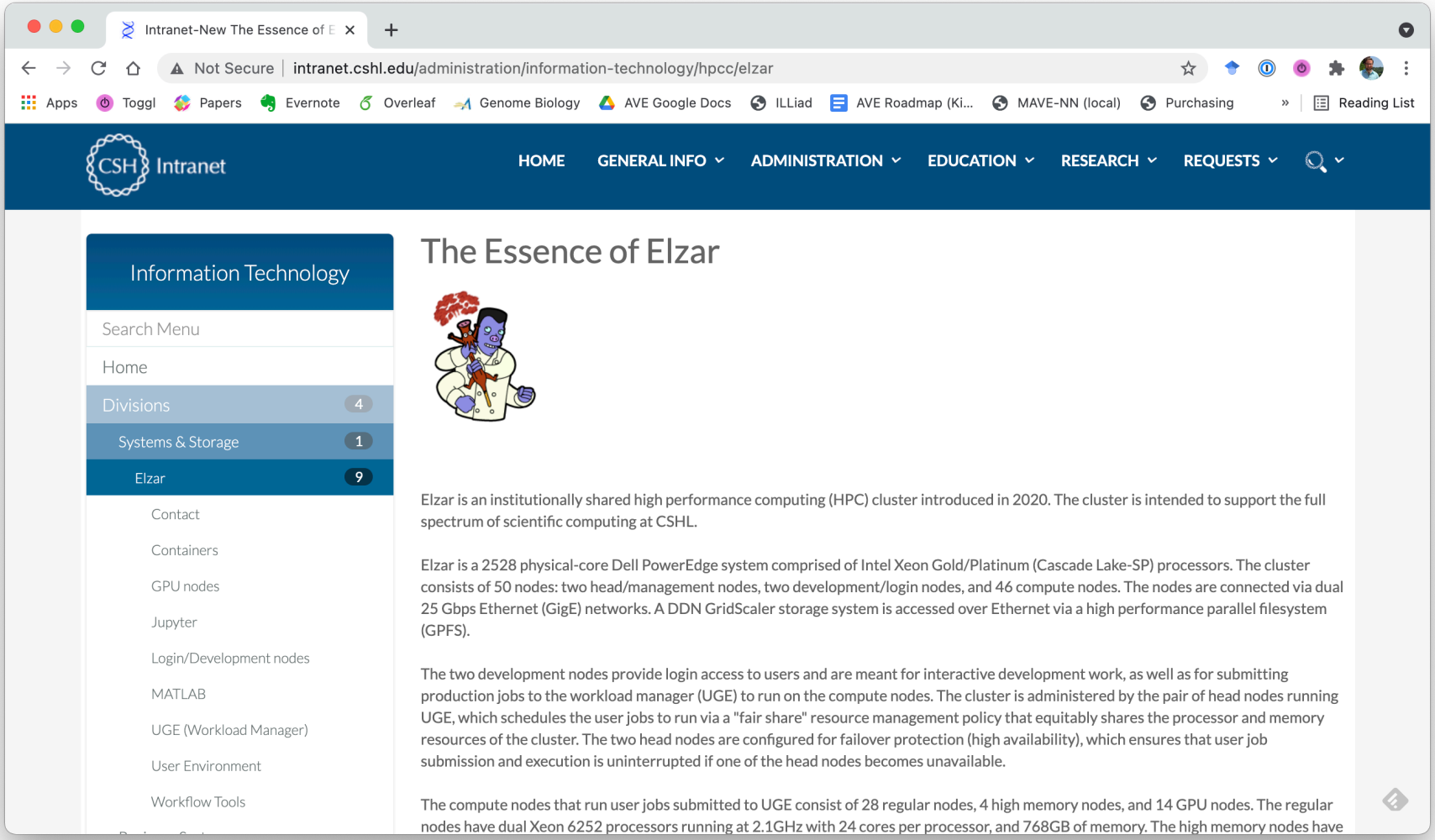
chromosome **window** **# reads**

local analysis
(python)



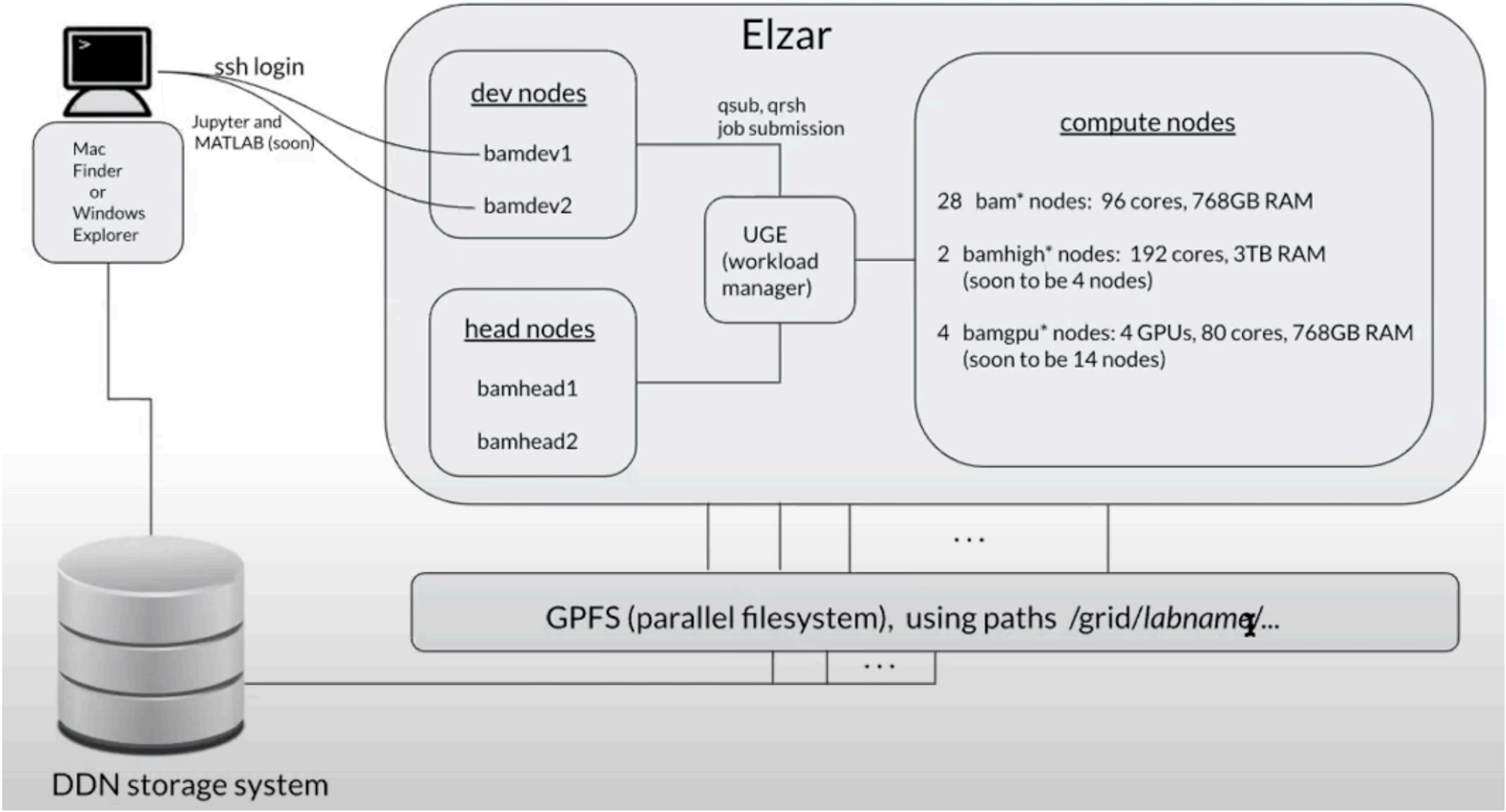
Elzar is the CSHL's high-performance computer cluster (HPCC)

http://intranet.cshl.edu/administration/information-technology/hpcc/elzar



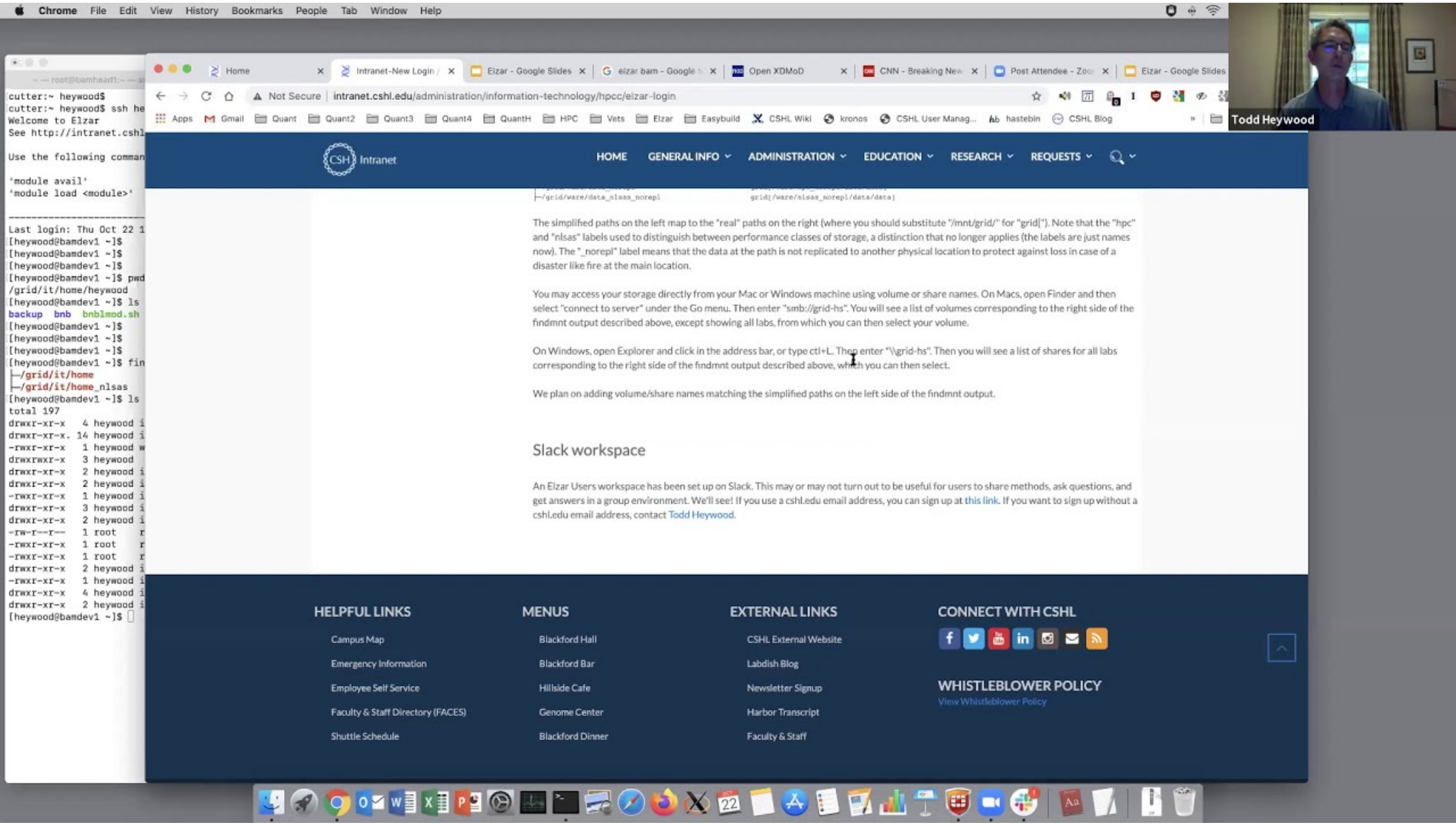
If you use Elzar ***at all*** for your work, please cite "NIH Grant S10OD028632-01"

Architecture of Elzar

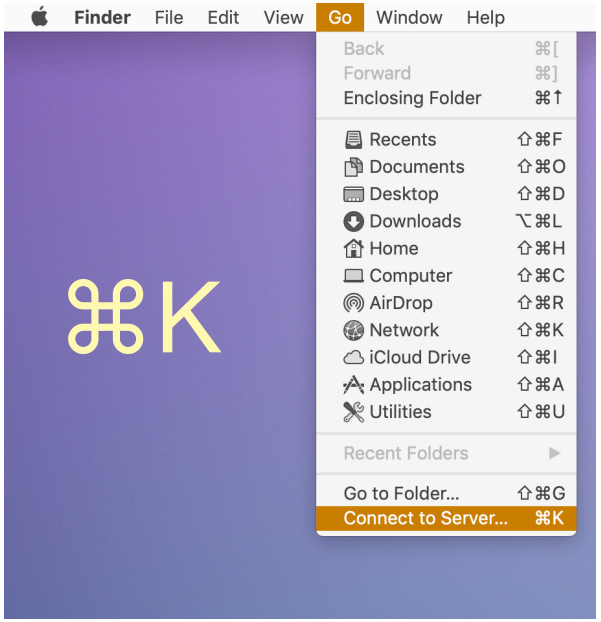


Elzar tutorial on YouTube

https://www.youtube.com/embed/D3wfhM_cQPY

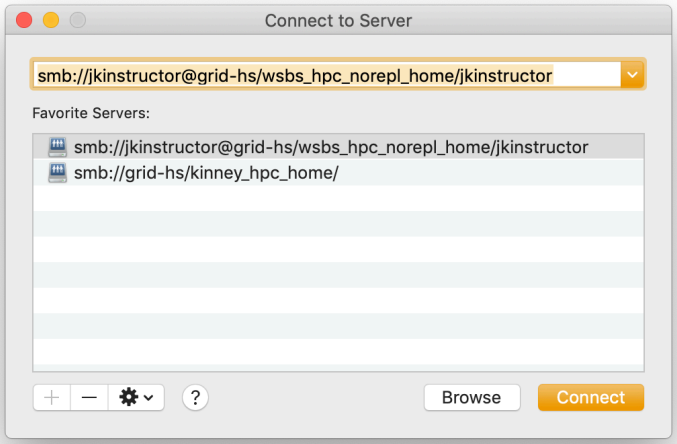


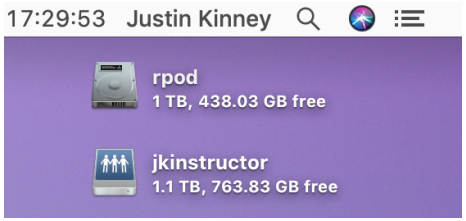
The Elzar disk can be mounted using smb

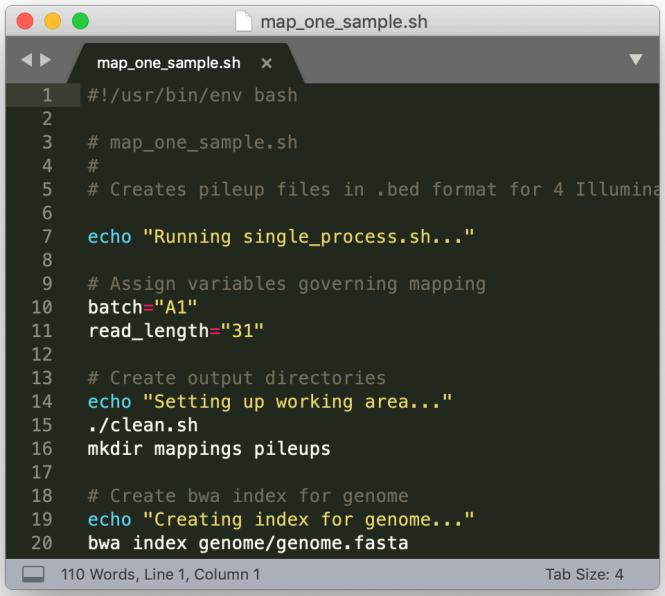


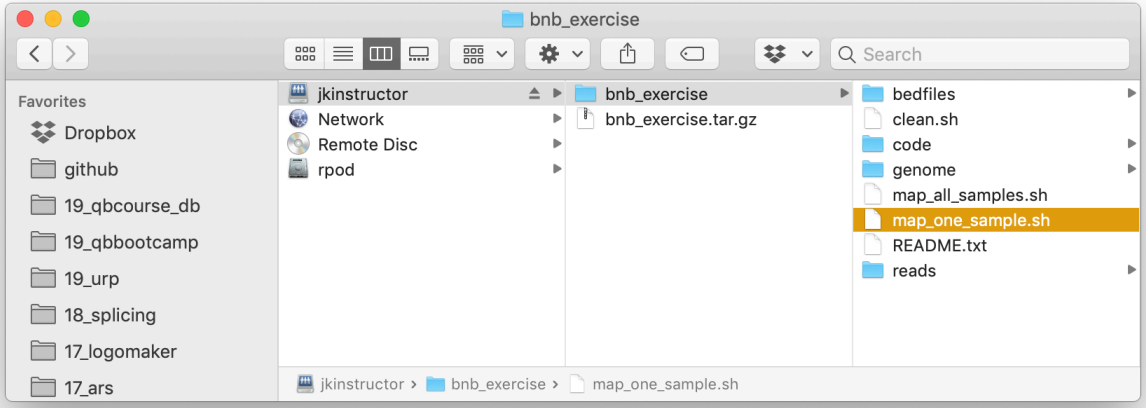
smb://you@grid-hs/wsbs_hpc_norepl_home/you

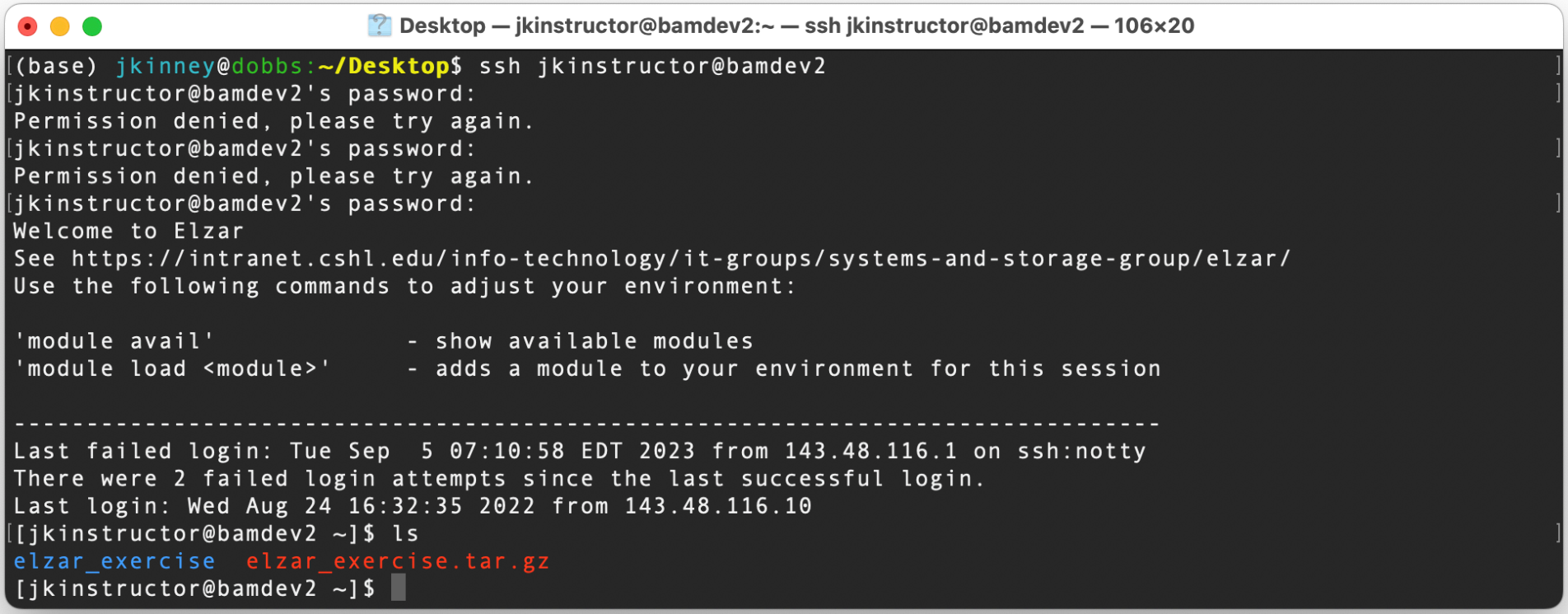
or smb://you@grid-hs/yourlab_hpc_home/you

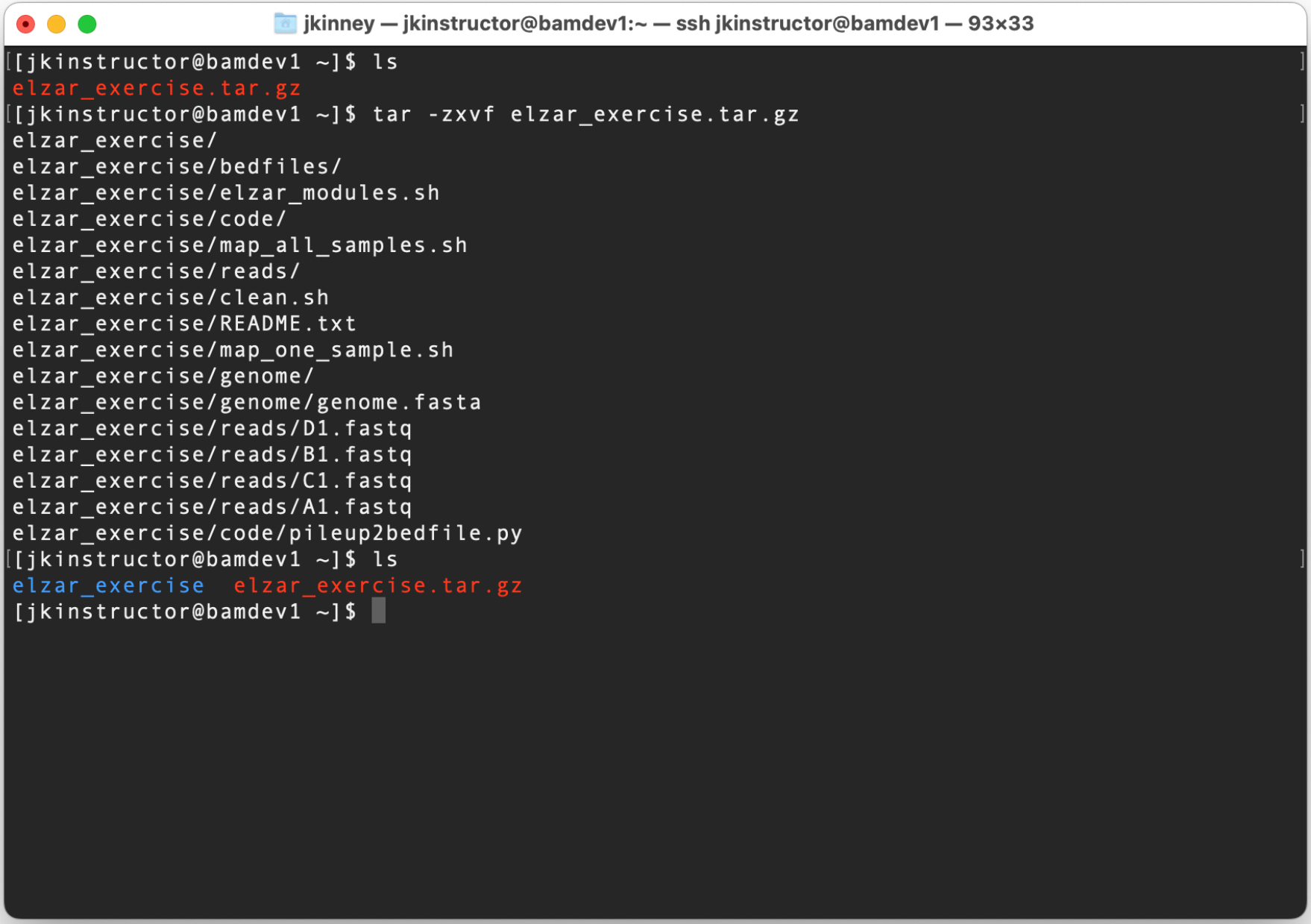


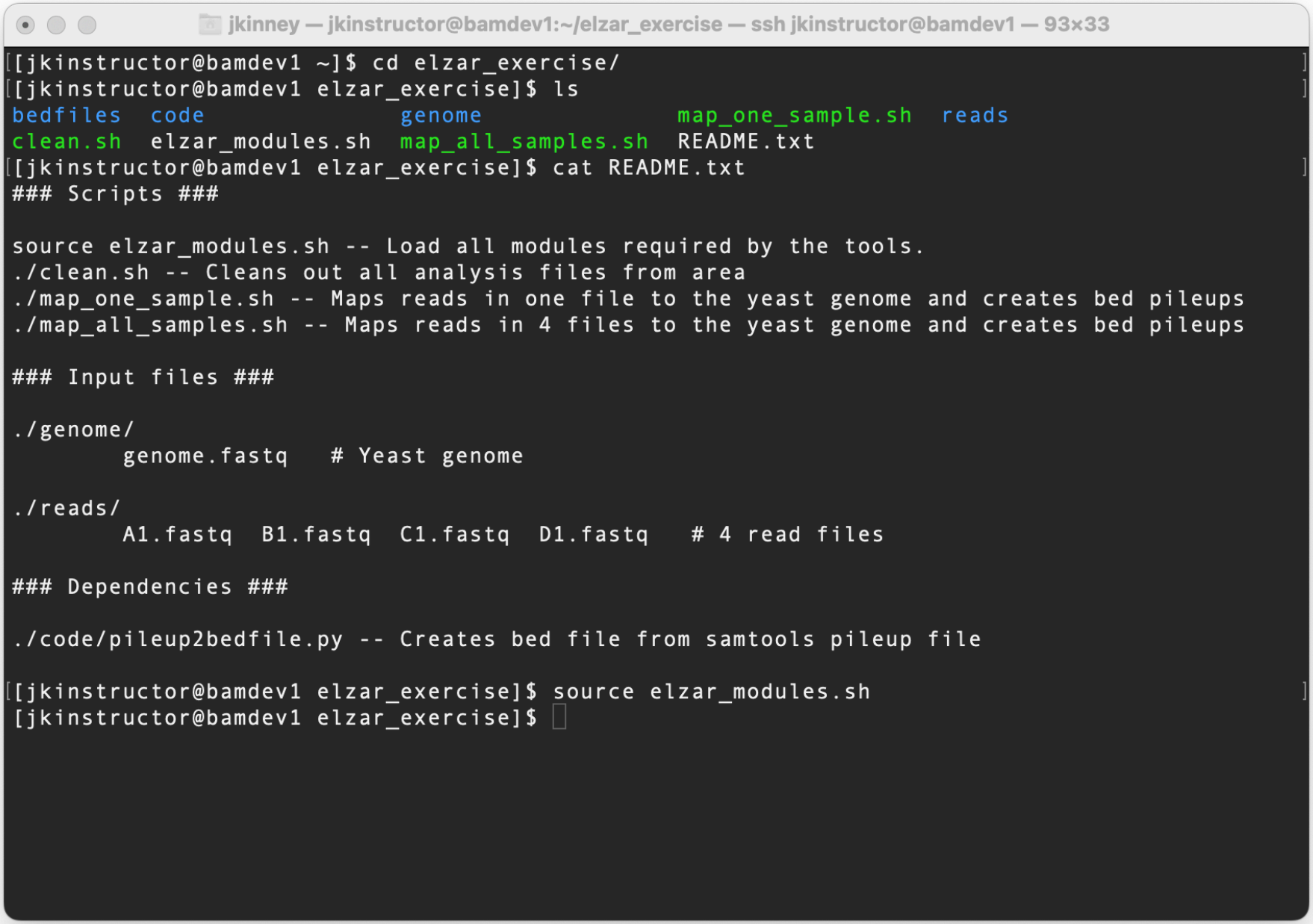


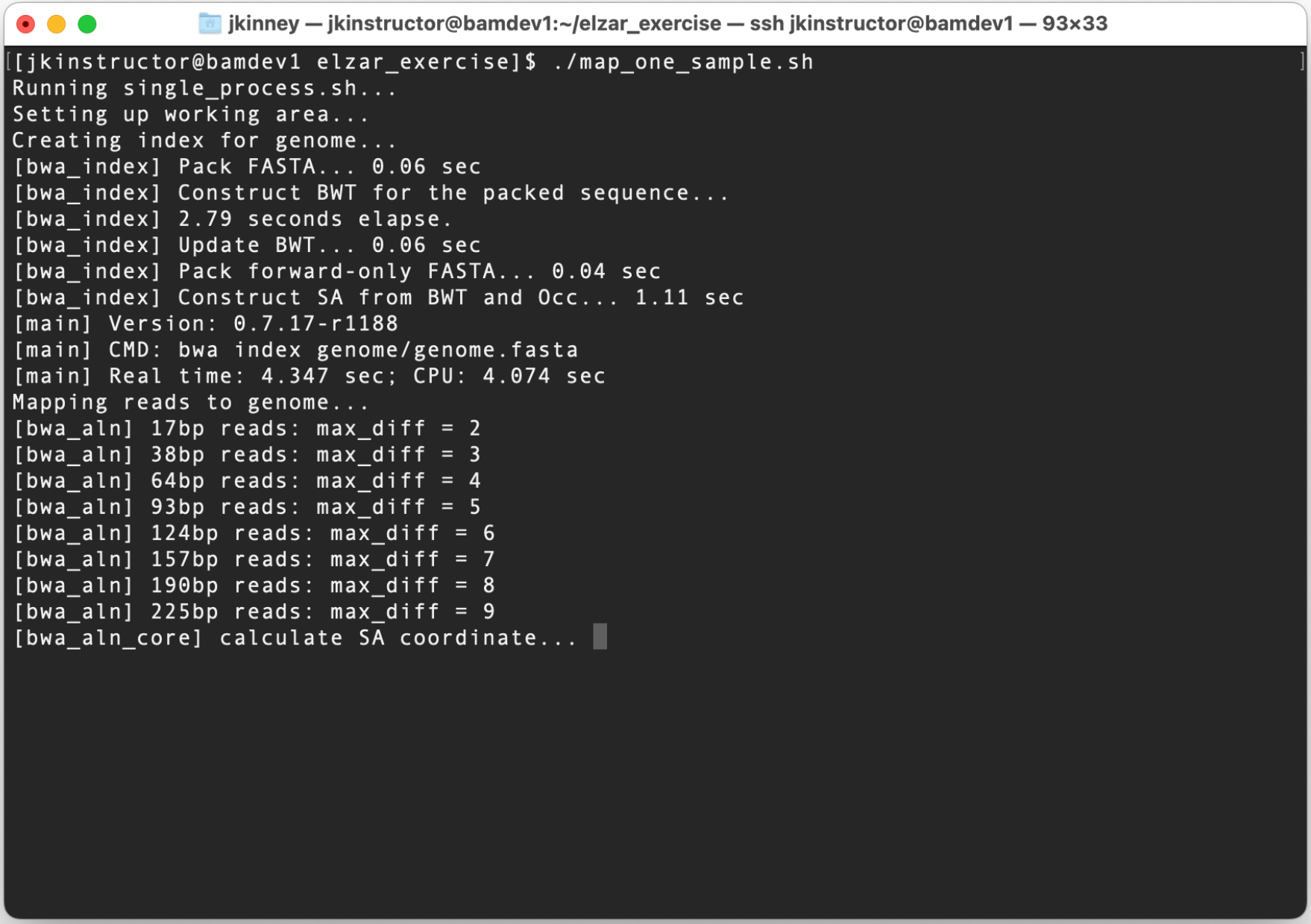


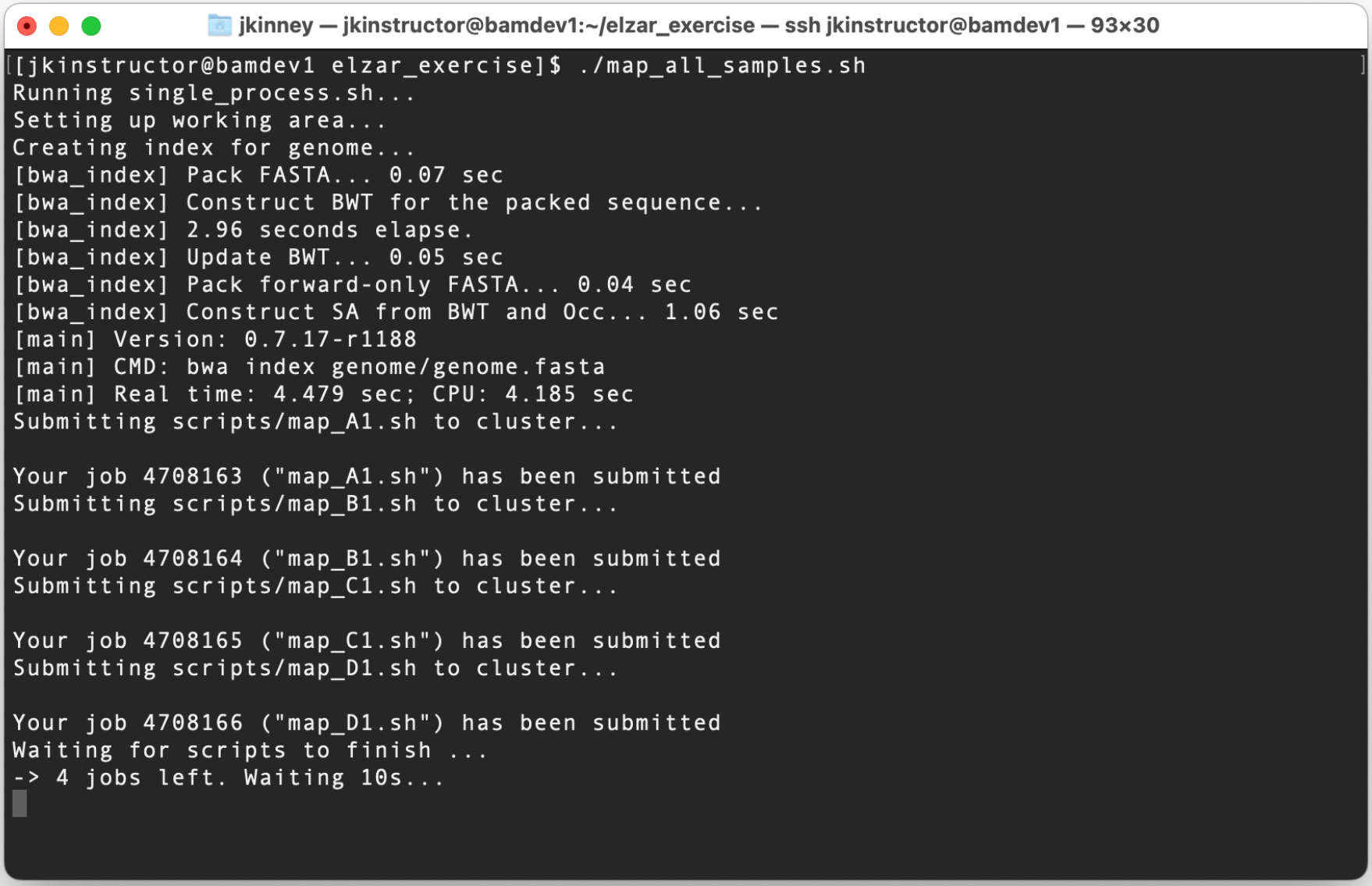


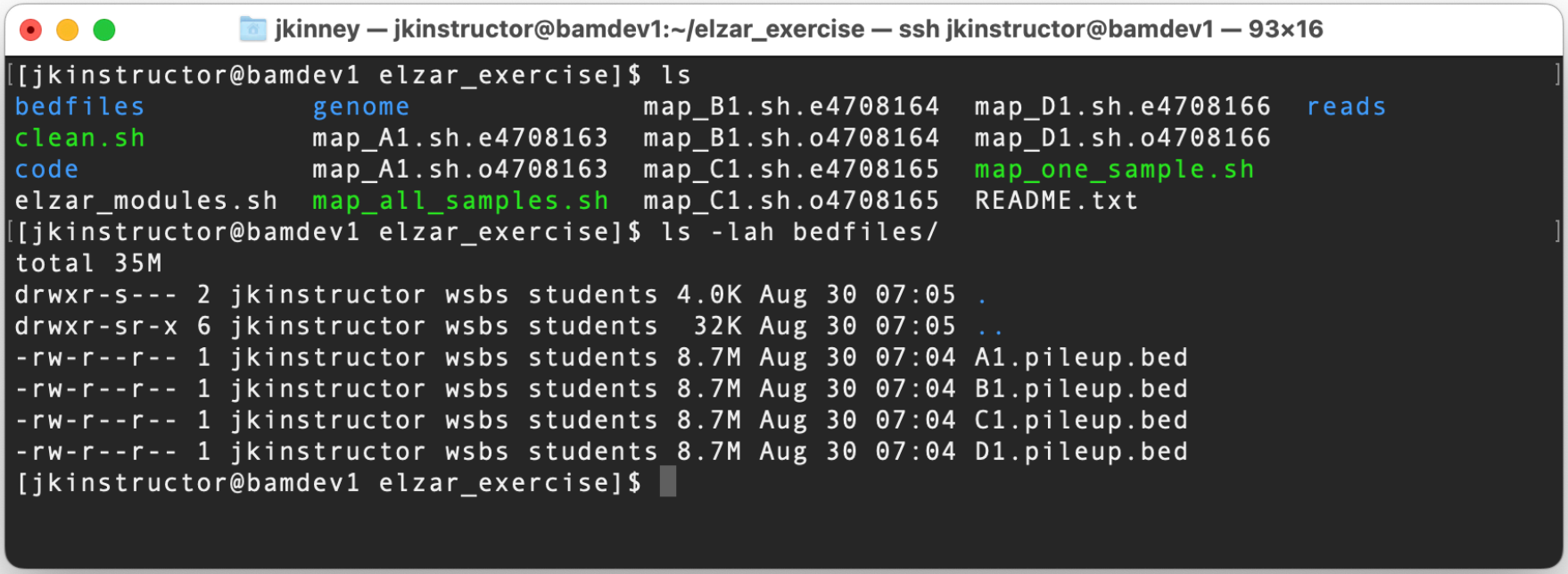

Desktop — jkinstructor@bamdev2:~ — ssh jkinstructor@bamdev2 — 106×20
(base) jkinney@dobbs:~/Desktop$ ssh jkinstructor@bamdev2
jkinstructor@bamdev2's password:
Permission denied, please try again.
jkinstructor@bamdev2's password:
Permission denied, please try again.
jkinstructor@bamdev2's password:
Welcome to Elzar
See https://intranet.cshl.edu/info-technology/it-groups/systems-and-storage-group/elzar/
Use the following commands to adjust your environment:
'module avail'   - show available modules
'module load <module>'   - adds a module to your environment for this session
Last failed login: Tue Sep 5 07:10:58 EDT 2023 from 143.48.116.1 on ssh:notty
There were 2 failed login attempts since the last successful login.
Last login: Wed Aug 24 16:32:35 2022 from 143.48.116.10
[jkinstructor@bamdev2 ~]$ ls
elzar_exercise elzar_exercise.tar.gz
[jkinstructor@bamdev2 ~]$


jkinney — jkinstructor@bamdev1:~ — ssh jkinstructor@bamdev1 — 93×33
[[jkinstructor@bamdev1 ~]$ ls
elzar_exercise.tar.gz
[[jkinstructor@bamdev1 ~]$ tar -zxvf elzar_exercise.tar.gz
elzar_exercise/
elzar_exercise/bedfiles/
elzar_exercise/elzar_modules.sh
elzar_exercise/code/
elzar_exercise/map_all_samples.sh
elzar_exercise/reads/
elzar_exercise/clean.sh
elzar_exercise/README.txt
elzar_exercise/map_one_sample.sh
elzar_exercise/genome/
elzar_exercise/genome/genome.fasta
elzar_exercise/reads/D1.fastq
elzar_exercise/reads/B1.fastq
elzar_exercise/reads/C1.fastq
elzar_exercise/reads/A1.fastq
elzar_exercise/code/pileup2bedfile.py
[[jkinstructor@bamdev1 ~]$ ls
elzar_exercise elzar_exercise.tar.gz
[jkinstructor@bamdev1 ~]$


jkinney — jkinstructor@bamdev1:~/elzar_exercise — ssh jkinstructor@bamdev1 — 93×33
[jkinstructor@bamdev1 ~]$ cd elzar_exercise/
[jkinstructor@bamdev1 elzar_exercise]$ ls
bedfiles code genome map_one_sample.sh reads
clean.sh elzar_modules.sh map_all_samples.sh README.txt
[jkinstructor@bamdev1 elzar_exercise]$ cat README.txt
Scripts
source elzar_modules.sh -- Load all modules required by the tools.
./clean.sh -- Cleans out all analysis files from area
./map_one_sample.sh -- Maps reads in one file to the yeast genome and creates bed pileups
./map_all_samples.sh -- Maps reads in 4 files to the yeast genome and creates bed pileups
Input files
./genome/
genome.fastq # Yeast genome
./reads/
A1.fastq B1.fastq C1.fastq D1.fastq # 4 read files
Dependencies
./code/pileup2bedfile.py -- Creates bed file from samtools pileup file
[jkinstructor@bamdev1 elzar_exercise]$ source elzar_modules.sh
[jkinstructor@bamdev1 elzar_exercise]$


jkinney — jkinstructor@bamdev1:~/elzar_exercise — ssh jkinstructor@bamdev1 — 93×33
[[jkinstructor@bamdev1 elzar_exercise]$ ./map_one_sample.sh]
Running single_process.sh...
Setting up working area...
Creating index for genome...
[bwa_index] Pack FASTA... 0.06 sec
[bwa_index] Construct BWT for the packed sequence...
[bwa_index] 2.79 seconds elapse.
[bwa_index] Update BWT... 0.06 sec
[bwa_index] Pack forward-only FASTA... 0.04 sec
[bwa_index] Construct SA from BWT and Occ... 1.11 sec
[main] Version: 0.7.17-r1188
[main] CMD: bwa index genome/genome.fasta
[main] Real time: 4.347 sec; CPU: 4.074 sec
Mapping reads to genome...
[bwa_aln] 17bp reads: max_diff = 2
[bwa_aln] 38bp reads: max_diff = 3
[bwa_aln] 64bp reads: max_diff = 4
[bwa_aln] 93bp reads: max_diff = 5
[bwa_aln] 124bp reads: max_diff = 6
[bwa_aln] 157bp reads: max_diff = 7
[bwa_aln] 190bp reads: max_diff = 8
[bwa_aln] 225bp reads: max_diff = 9
[bwa_aln_core] calculate SA coordinate...


jkinney — jkinstructor@bamdev1:~/elzar_exercise — ssh jkinstructor@bamdev1 — 93×30
[jkinstructor@bamdev1 elzar_exercise]$ ./map_all_samples.sh
Running single_process.sh...
Setting up working area...
Creating index for genome...
[bwa_index] Pack FASTA... 0.07 sec
[bwa_index] Construct BWT for the packed sequence...
[bwa_index] 2.96 seconds elapse.
[bwa_index] Update BWT... 0.05 sec
[bwa_index] Pack forward-only FASTA... 0.04 sec
[bwa_index] Construct SA from BWT and Occ... 1.06 sec
[main] Version: 0.7.17-r1188
[main] CMD: bwa index genome/genome.fasta
[main] Real time: 4.479 sec; CPU: 4.185 sec
Submitting scripts/map_A1.sh to cluster...
Your job 4708163 ("map_A1.sh") has been submitted
Submitting scripts/map_B1.sh to cluster...
Your job 4708164 ("map_B1.sh") has been submitted
Submitting scripts/map_C1.sh to cluster...
Your job 4708165 ("map_C1.sh") has been submitted
Submitting scripts/map_D1.sh to cluster...
Your job 4708166 ("map_D1.sh") has been submitted
Waiting for scripts to finish ...
-> 4 jobs left. Waiting 10s...


jkinney — jkinstructor@bamdev1:~/elzar_exercise — ssh jkinstructor@bamdev1 — 93×16
[jkinstructor@bamdev1 elzar_exercise]$ ls
bedfiles genome map_B1.sh.e4708164 map_D1.sh.e4708166 reads
clean.sh map_A1.sh.e4708163 map_B1.sh.o4708164 map_D1.sh.o4708166
code map_A1.sh.o4708163 map_C1.sh.e4708165 map_one_sample.sh
elzar_modules.sh map_all_samples.sh map_C1.sh.o4708165 README.txt
[jkinstructor@bamdev1 elzar_exercise]$ ls -lah bedfiles/
total 35M
drwxr-s--- 2 jkinstructor wsbs students 4.0K Aug 30 07:05 .
drwxr-sr-x 6 jkinstructor wsbs students 32K Aug 30 07:05 ..
-rw-r--r-- 1 jkinstructor wsbs students 8.7M Aug 30 07:04 A1.pileup.bed
-rw-r--r-- 1 jkinstructor wsbs students 8.7M Aug 30 07:04 B1.pileup.bed
-rw-r--r-- 1 jkinstructor wsbs students 8.7M Aug 30 07:04 C1.pileup.bed
-rw-r--r-- 1 jkinstructor wsbs students 8.7M Aug 30 07:04 D1.pileup.bed
[jkinstructor@bamdev1 elzar_exercise]$

To do this morning:

1. Copy **elzar_exercises.tar.gz** from **22e_qbbootcamp/** to your home directory on Elzar

2. Map one sample of reads to genome using **map_one_sample.sh**

3. Submit four mapping jobs to cluster using **map_all_samples.sh**

4. Copy .bed files to local machine

5. This afternoon: Visualize replication profiles using Python.