Assignment_2_FastQC_Working_Notes

Helpful

For all steps below, process the two libraries separately.

My data assignments are:
Grace 15_3C_mbnl_S11_L008 17_3E_fox_S13_L008
Henceforth referred to as 15 and 17.

Files found here:

```
/projects/bgmp/shared/2017_sequencing/demultiplexed/
# Specific paths:
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R1_001
.fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R2_001
.fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R1_001.
fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R2_001.
fastq.gz
```

Repo here:

```
/projects/bgmp/ghach/bioinfo/Bi623/QAA
```

Tutorials for command line interface

https://hbctraining.github.io/Intro-to-rnaseq-hpc-salmon-flipped/lessons/05_qc_running_fastqc_interactively.html#:~:text=To%20run%20the%20FastQC%20program,the%20command%20to%20run%20FastQC.
fastqc seqfile1, seqfile2

Part 1

Creating environment, installing fast QC

```
conda create --name QAA
conda activate QAA
conda install bioconda::fastqc
```

Answered yes to all prompts, verified version:

```
fastqc --version
```

Output: FastQC v0.12.1

Parameters to run fast QC

--extract If set then the zipped output file will be uncompressed in
the same directory after it has been created. If --delete is
also specified then the zip file will be removed after the
contents are unzipped.
Set these both to true?

--o output directory

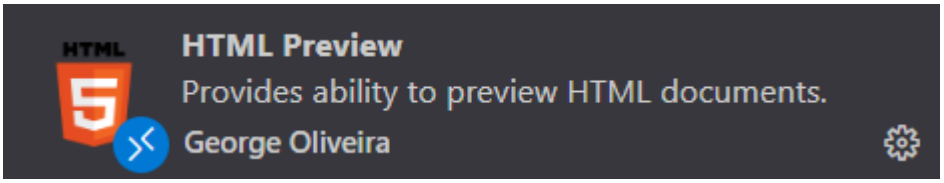
--svg Save the graphs in the report in SVG format.
Set to true, I like svgs

-a Specifies a non-default file which contains the list of
--adapters adapter sequences which will be explicity searched against
the library. The file must contain sets of named adapters
in the form name[tab]sequence. Lines prefixed with a hash
will be ignored.
If this isn't relevant now, it will be at some point

Ran this command:

```
fastqc
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R1_001
.fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R2_001
.fastq.gz -o output
```

Should specify delete option to delete intermediate zip files.
I don't know how to deal with html files here. I'm going to try an extension:



This works.
Control shift v to turn html file into a preview, same command to toggle off.
For the sake of experimentation, I'm also going to make folder test_output and run the same command with altered parameters:

```
fastqc
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R1_001
.fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R2_001
.fastq.gz -o test_output --svg --extract --delete
```

^ This is the better way to do it. unzips everything into a nice folder

Final commands used:

```
mkdir -p 15_output
mkdir -p 17_output
fastqc
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R1_001
.fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R2_001
.fastq.gz -o 15_output --svg --extract --delete
fastqc
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R1_001.
fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R2_001.
fastq.gz
```

Realized QAA has nothing in it. Remedied by activating environment:

```
conda install python=3.12
pip install matplotlib
```

```
pip install numpy # this didn't work because numpy comes along with matplotlib
```

Added sbatch scripts run_demux_plotting.sh run_fastqc.sh.
Stupid how fast the latter is compared to the former. Note that fastQC is coded in Java, generally a lot faster than python, and also likely optimized (taking the name on faith)

Part 2

```
conda install bioconda::cutadapt
cutadapt --version
conda install bioconda::trimmomatic
trimmomatic -version
```

Proceeded by selecting y, versions are correct.

Run requires naming the adapters:

```
For paired-end reads:
        cutadapt -a ADAPT1 -A ADAPT2 [options] -o out1.fastq -p out2.fastq
in1.fastq in2.fastq

Replace "ADAPTER" with the actual sequence of your 3' adapter. IUPAC wildcard
characters are supported. All reads from input.fastq will be written to
output.fastq with the adapter sequence removed. Adapter matching is
error-tolerant. Multiple adapter sequences can be given (use further -a
options), but only the best-matching adapter will be removed.
```

Hunting for adapters

from:
https://support-docs.illumina.com/SHARE/AdapterSequences/Content/SHARE/AdapterSeq/Illumina_DNA/IlluminaUDIndexes.htm
We get these:
Index 1 (i7) Adapters -> affixed to R2
CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG
Index 2 (i5) Adapters
AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC

But grepping these left me empty-handed because the reverse complement is what's actually sequenced.

R2 Actual Adapter Sequence:

- GTGTAGATCTCGGTGGTCGCCGTATCATT
  *R1 Actual Adapter Sequence:
- ATCTCGTATGCCGTCTTCTGCTTG

but IDK which of these you use for cutadapt.
First, trying above sequences:

```
cutadapt -a ATCTCGTATGCCGTCTTCTGCTTG -A GTGTAGATCTCGGTGGTCGCCGTATCATT -o
17_R1_adapters_removed.fastq -p 17_R2_adapters_removed.fastq
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R1_001.
fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R2_001.
fastq.gz
```

hmm the actual number of sequences trimmed is paltry and my sequences don't match the ones in the assignment description.

Eventually found the correct adapters here:
https://dnatech.genomecenter.ucdavis.edu/wp-content/uploads/2019/03/illumina-adapter-sequences-2019-1000000002694-10.pdf

IDT for Illumina TruSeq DNA and RNA UD Indexes

These unique dual (UD) index adapters are arranged in the plate to enforce the recommended pairing strategy.

Adapter Trimming

The following sequences are used for adapter trimming.

Read 1

AGATCGGAAGAGCACACGTCTGAACTCCAGTCA

Read 2

AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

Index Adapters

Index 1 (i7) Adapters

GATCGGAAGAGCACACGTCTGAACTCCAGTCAC[i7]ATCTCGTATGCCGTCTTCTGCTTG

Index 2 (i5) Adapters

AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT

**Adapter trimming happens only at the 3' end! The adapters on the 5' end aren't sequenced, because the sequencing starts at the first base of the DNA insert. IFF the insert is too short, does some of the adapter on the opposite end get sequenced, that's wat we're removing.

Rerunning:

```
cutadapt -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA -A
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT -o 17_R1_adapters_removed.fastq -p
17_R2_adapters_removed.fastq
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R1_001.
fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/17_3E_fox_S13_L008_R2_001.
fastq.gz
```

Output:

```
Finished in 59.628 s (5.060 µs/read; 11.86 M reads/minute).

=== Summary ===

Total read pairs processed:         11,784,410
```

```
  Read 1 with adapter:                1,024,588 (8.7%)
  Read 2 with adapter:                1,104,503 (9.4%)
Pairs written (passing filters):    11,784,410 (100.0%)

Total basepairs processed: 2,380,450,820 bp
  Read 1: 1,190,225,410 bp
  Read 2: 1,190,225,410 bp
Total written (filtered):  2,335,751,295 bp (98.1%)
  Read 1: 1,168,027,279 bp
  Read 2: 1,167,724,016 bp

=== First read: Adapter 1 ===

Sequence: AGATCGGAAGAGCACACGTCTGAACTCCAGTCA; Type: regular 3'; Length: 33;
Trimmed: 1024588 times

Minimum overlap: 3
No. of allowed errors:
1-9 bp: 0; 10-19 bp: 1; 20-29 bp: 2; 30-33 bp: 3

Bases preceding removed adapters:
  A: 16.1%
  C: 29.9%
  G: 34.4%
  T: 13.1%
  none/other: 6.4%

Overview of removed sequences
length  count   expect  max.err error counts
3       225955  184131.4        0       225955
4       66666   46032.9 0       66666
5       35777   11508.2 0       35777
6       25568   2877.1  0       25568
7       24280   719.3   0       24280
8       23497   179.8   0       23497
9       23284   45.0    0       23009 275
10      23486   11.2    1       22633 853
11      22250   2.8     1       21493 757
12      22031   0.7     1       21267 764
13      21493   0.2     1       20780 713
```

```
14      20992   0.0     1       20277 715
15      20729   0.0     1       19970 759
16      20104   0.0     1       19316 788
17      19444   0.0     1       18610 834
18      18735   0.0     1       17954 761 20
19      17917   0.0     1       17041 840 36
20      17612   0.0     2       16716 789 107
21      16784   0.0     2       15947 745 92
22      16414   0.0     2       15556 749 109
23      16116   0.0     2       15207 805 104
24      15528   0.0     2       14709 716 103
25      15233   0.0     2       14396 735 102
26      14526   0.0     2       13654 772 100
27      13947   0.0     2       13057 770 110 10
28      13344   0.0     2       12519 718 95 12
29      12508   0.0     2       11698 695 104 11
30      12041   0.0     3       11191 707 105 38
31      11601   0.0     3       10771 714 84 32
32      10994   0.0     3       10186 650 104 54
33      10295   0.0     3       9573 584 105 33
34      9834    0.0     3       9170 543 92 29
35      9290    0.0     3       8657 527 76 30
36      8851    0.0     3       8263 477 88 23
37      8509    0.0     3       7954 478 61 16
38      8200    0.0     3       7673 443 57 27
39      7677    0.0     3       7192 412 45 28
40      7305    0.0     3       6858 392 45 10
41      6616    0.0     3       6218 344 38 16
42      6034    0.0     3       5664 325 38 7
43      5472    0.0     3       5140 284 35 13
44      4932    0.0     3       4638 266 23 5
45      4696    0.0     3       4393 273 24 6
46      4063    0.0     3       3826 202 29 6
47      3834    0.0     3       3607 191 24 12
48      3522    0.0     3       3326 164 25 7
49      3301    0.0     3       3076 190 28 7
50      3044    0.0     3       2873 154 14 3
51      2647    0.0     3       2492 133 19 3
52      2488    0.0     3       2350 117 14 7
53      2071    0.0     3       1937 111 14 9
```

```
54      1881    0.0     3       1783 84 11 3
55      1615    0.0     3       1525 75 14 1
56      1351    0.0     3       1279 65 6 1
57      1387    0.0     3       1306 73 8
58      1214    0.0     3       1143 65 2 4
59      1099    0.0     3       1032 55 11 1
60      1039    0.0     3       978 49 10 2
61      963     0.0     3       911 37 14 1
62      912     0.0     3       864 40 7 1
63      773     0.0     3       721 43 7 2
64      683     0.0     3       641 36 4 2
65      547     0.0     3       513 27 6 1
66      527     0.0     3       494 27 5 1
67      456     0.0     3       433 18 5
68      445     0.0     3       425 20
69      408     0.0     3       383 23 1 1
70      372     0.0     3       353 18 0 1
71      330     0.0     3       311 14 1 4
72      297     0.0     3       285 10 2
73      245     0.0     3       233 10 2
74      206     0.0     3       190 11 5
75      157     0.0     3       141 14 2
76      103     0.0     3       91 11 1
77      85      0.0     3       81 3 1
78      75      0.0     3       66 9
79      75      0.0     3       66 7 1 1
80      28      0.0     3       25 1 2
81      34      0.0     3       32 2
82      15      0.0     3       14 1
83      19      0.0     3       18 1
84      14      0.0     3       14
85      21      0.0     3       19 1 1
86      8       0.0     3       8
87      17      0.0     3       17
88      12      0.0     3       12
89      25      0.0     3       24 1
90      11      0.0     3       10 0 0 1
91      17      0.0     3       14 2 1
92      5       0.0     3       4 0 0 1
93      12      0.0     3       11 1
```

```
94      9       0.0     3       6 3
95      13      0.0     3       13
96      13      0.0     3       12 1
97      4       0.0     3       4
98      12      0.0     3       11 0 1
99      5       0.0     3       5
100     10      0.0     3       9 1
101     65502   0.0     3       6 58739 6317 440


=== Second read: Adapter 2 ===

Sequence: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT; Type: regular 3'; Length: 33;
Trimmed: 1104503 times

Minimum overlap: 3
No. of allowed errors:
1-9 bp: 0; 10-19 bp: 1; 20-29 bp: 2; 30-33 bp: 3

Bases preceding removed adapters:
  A: 16.3%
  C: 32.8%
  G: 37.0%
  T: 7.9%
  none/other: 5.9%

Overview of removed sequences
length  count   expect  max.err error counts
3       287280  184131.4        0       287280
4       79052   46032.9 0       79052
5       37530   11508.2 0       37530
6       26927   2877.1  0       26927
7       24555   719.3   0       24555
8       23609   179.8   0       23609
9       23651   45.0    0       23225 426
10      23709   11.2    1       22832 877
11      22464   2.8     1       21739 725
12      22184   0.7     1       21611 573
13      21583   0.2     1       21051 532
14      21079   0.0     1       20572 507
```

```
15      20784   0.0     1       20143 641
16      20170   0.0     1       19626 544
17      19532   0.0     1       18908 624
18      18815   0.0     1       18062 746 7
19      17998   0.0     1       17386 599 13
20      17658   0.0     2       16908 669 81
21      16843   0.0     2       16105 657 81
22      16499   0.0     2       15813 622 64
23      16152   0.0     2       15491 578 83
24      15590   0.0     2       14847 663 80
25      15271   0.0     2       14511 663 97
26      14568   0.0     2       13851 622 95
27      14001   0.0     2       13356 540 103 2
28      13388   0.0     2       12746 553 85 4
29      12565   0.0     2       11905 564 88 8
30      12075   0.0     3       11447 536 69 23
31      11623   0.0     3       10739 743 108 33
32      11044   0.0     3       10417 493 108 26
33      10317   0.0     3       9727 473 78 39
34      9869    0.0     3       9297 451 85 36
35      9309    0.0     3       8797 424 65 23
36      8884    0.0     3       8408 384 61 31
37      8533    0.0     3       8029 408 62 34
38      8221    0.0     3       7735 375 66 45
39      7687    0.0     3       7255 339 65 28
40      7338    0.0     3       6925 324 60 29
41      6627    0.0     3       6298 259 38 32
42      6044    0.0     3       5720 261 42 21
43      5480    0.0     3       5196 235 26 23
44      4966    0.0     3       4700 210 36 20
45      4708    0.0     3       4444 220 28 16
46      4080    0.0     3       3892 155 24 9
47      3857    0.0     3       3650 166 20 21
48      3536    0.0     3       3332 175 21 8
49      3326    0.0     3       3154 128 26 18
50      3066    0.0     3       2890 143 25 8
51      2675    0.0     3       2514 128 20 13
52      2511    0.0     3       2380 104 21 6
53      2083    0.0     3       1971 89 13 10
54      1913    0.0     3       1803 85 11 14
```

```
55      1635    0.0     3       1506 102 18 9
56      1379    0.0     3       1292 71 11 5
57      1409    0.0     3       1317 68 17 7
58      1241    0.0     3       1168 59 10 4
59      1126    0.0     3       1043 62 13 8
60      1054    0.0     3       992 54 4 4
61      982     0.0     3       912 50 9 11
62      933     0.0     3       863 51 10 9
63      790     0.0     3       739 34 7 10
64      704     0.0     3       656 32 12 4
65      566     0.0     3       516 38 8 4
66      543     0.0     3       503 33 6 1
67      472     0.0     3       434 28 8 2
68      463     0.0     3       432 25 4 2
69      427     0.0     3       396 19 10 2
70      386     0.0     3       356 17 9 4
71      349     0.0     3       314 23 6 6
72      312     0.0     3       283 22 3 4
73      257     0.0     3       233 16 5 3
74      215     0.0     3       190 20 3 2
75      169     0.0     3       156 9 3 1
76      111     0.0     3       100 8 1 2
77      95      0.0     3       83 9 2 1
78      82      0.0     3       70 9 2 1
79      78      0.0     3       73 1 3 1
80      30      0.0     3       26 2 2
81      38      0.0     3       33 1 3 1
82      16      0.0     3       15 1
83      19      0.0     3       18 1
84      16      0.0     3       15 0 0 1
85      23      0.0     3       21 1 1
86      7       0.0     3       6 1
87      18      0.0     3       15 2 1
88      12      0.0     3       8 4
89      24      0.0     3       22 2
90      10      0.0     3       9 1
91      17      0.0     3       14 2 1
92      4       0.0     3       4
93      12      0.0     3       11 1
94      12      0.0     3       6 3 0 3
```

```
95      13      0.0     3       10 3
96      15      0.0     3       10 2 1 2
97      6       0.0     3       2 2 1 1
98      13      0.0     3       8 2 3
99      5       0.0     3       4 0 1
100     10      0.0     3       1 5 2 2
101     65176   0.0     3       6 58156 6462 552
```

Running:

```
cutadapt -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA -A
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT -o 15_R1_adapters_removed.fastq -p
15_R2_adapters_removed.fastq
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R1_001
.fastq.gz
/projects/bgmp/shared/2017_sequencing/demultiplexed/15_3C_mbnl_S11_L008_R2_001
.fastq.gz
```

Output:

```
Processing paired-end reads on 1 core ...
Done           00:00:38     7,806,403 reads @   4.9 µs/read;  12.29 M
reads/minute
Finished in 38.104 s (4.881 µs/read; 12.29 M reads/minute).

=== Summary ===

Total read pairs processed:          7,806,403
  Read 1 with adapter:                 417,810 (5.4%)
  Read 2 with adapter:                 477,359 (6.1%)
Pairs written (passing filters):     7,806,403 (100.0%)

Total basepairs processed: 1,576,893,406 bp
  Read 1:   788,446,703 bp
  Read 2:   788,446,703 bp
Total written (filtered):  1,566,917,010 bp (99.4%)
  Read 1:   783,587,227 bp
  Read 2:   783,329,783 bp

=== First read: Adapter 1 ===
```

```
Sequence: AGATCGGAAGAGCACACGTCTGAACTCCAGTCA; Type: regular 3'; Length: 33;
Trimmed: 417810 times

Minimum overlap: 3
No. of allowed errors:
1-9 bp: 0; 10-19 bp: 1; 20-29 bp: 2; 30-33 bp: 3

Bases preceding removed adapters:
  A: 17.0%
  C: 29.2%
  G: 37.9%
  T: 15.8%
  none/other: 0.2%

Overview of removed sequences
length  count   expect  max.err error counts
3       152119  121975.0        0       152119
4       40634   30493.8 0       40634
5       18152   7623.4  0       18152
6       11433   1905.9  0       11433
7       10612   476.5   0       10612
8       9967    119.1   0       9967
9       9897    29.8    0       9695 202
10      9771    7.4     1       9375 396
11      9272    1.9     1       8950 322
12      8958    0.5     1       8669 289
13      8435    0.1     1       8183 252
14      7935    0.0     1       7658 277
15      7804    0.0     1       7549 255
16      7350    0.0     1       7094 256
17      7259    0.0     1       6983 276
18      6676    0.0     1       6426 245 5
19      6321    0.0     1       6049 264 8
20      5859    0.0     2       5566 261 32
21      5824    0.0     2       5576 220 28
22      5455    0.0     2       5191 227 37
23      5009    0.0     2       4748 227 34
24      4769    0.0     2       4530 211 28
25      4490    0.0     2       4245 216 29
```

```
26      4138    0.0     2       3888 210 40
27      3984    0.0     2       3763 197 23 1
28      3806    0.0     2       3583 197 24 2
29      3369    0.0     2       3158 184 26 1
30      3224    0.0     3       3015 173 23 13
31      2883    0.0     3       2713 136 24 10
32      2616    0.0     3       2464 123 19 10
33      2510    0.0     3       2359 130 14 7
34      2256    0.0     3       2110 124 15 7
35      2010    0.0     3       1886 107 13 4
36      1944    0.0     3       1835 93 13 3
37      1836    0.0     3       1717 95 16 8
38      1672    0.0     3       1577 82 10 3
39      1575    0.0     3       1486 77 9 3
40      1355    0.0     3       1283 60 9 3
41      1188    0.0     3       1123 58 5 2
42      1074    0.0     3       1024 45 4 1
43      986     0.0     3       927 50 6 3
44      898     0.0     3       850 38 9 1
45      829     0.0     3       781 45 3
46      821     0.0     3       770 43 5 3
47      696     0.0     3       663 26 6 1
48      692     0.0     3       641 40 8 3
49      663     0.0     3       627 28 7 1
50      571     0.0     3       537 28 3 3
51      497     0.0     3       459 31 7
52      456     0.0     3       431 18 5 2
53      437     0.0     3       416 15 5 1
54      351     0.0     3       322 26 1 2
55      362     0.0     3       338 18 4 2
56      303     0.0     3       289 10 2 2
57      313     0.0     3       297 12 3 1
58      279     0.0     3       263 12 3 1
59      261     0.0     3       243 16 2
60      268     0.0     3       258 5 2 3
61      239     0.0     3       228 7 4
62      196     0.0     3       180 14 1 1
63      216     0.0     3       197 16 3
64      197     0.0     3       186 11
65      152     0.0     3       142 9 0 1
```

66	155	0.0	3	146 5 2 2
67	122	0.0	3	121 1
68	120	0.0	3	112 5 2 1
69	92	0.0	3	85 6 1
70	84	0.0	3	81 0 2 1
71	68	0.0	3	66 2
72	83	0.0	3	80 3
73	70	0.0	3	65 3 1 1
74	50	0.0	3	46 4
75	31	0.0	3	29 0 1 1
76	29	0.0	3	23 4 2
77	15	0.0	3	14 1
78	13	0.0	3	12 1
79	15	0.0	3	15
80	6	0.0	3	6
81	6	0.0	3	6
82	4	0.0	3	3 1
83	7	0.0	3	6 1
84	1	0.0	3	1
85	6	0.0	3	6
86	5	0.0	3	5
87	2	0.0	3	2
88	1	0.0	3	1
89	2	0.0	3	2
90	2	0.0	3	2
91	3	0.0	3	3
92	3	0.0	3	3
93	1	0.0	3	1
95	2	0.0	3	2
96	2	0.0	3	1 1
101	686	0.0	3	2 600 81 3

=== Second read: Adapter 2 ===

Sequence: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT; Type: regular 3'; Length: 33; Trimmed: 477359 times

Minimum overlap: 3
No. of allowed errors:

```
1-9 bp: 0; 10-19 bp: 1; 20-29 bp: 2; 30-33 bp: 3

Bases preceding removed adapters:
  A: 19.7%
  C: 29.8%
  G: 41.0%
  T: 9.4%
  none/other: 0.1%

Overview of removed sequences
length  count   expect  max.err error counts
3       199425  121975.0        0       199425
4       47599   30493.8 0       47599
5       19744   7623.4  0       19744
6       12401   1905.9  0       12401
7       10799   476.5   0       10799
8       10053   119.1   0       10053
9       10101   29.8    0       9767 334
10      9962    7.4     1       9460 502
11      9490    1.9     1       9136 354
12      9057    0.5     1       8791 266
13      8483    0.1     1       8292 191
14      8005    0.0     1       7815 190
15      7863    0.0     1       7612 251
16      7412    0.0     1       7224 188
17      7300    0.0     1       7070 230
18      6732    0.0     1       6426 304 2
19      6370    0.0     1       6165 201 4
20      5896    0.0     2       5661 200 35
21      5856    0.0     2       5582 242 32
22      5504    0.0     2       5276 197 31
23      5056    0.0     2       4850 179 27
24      4802    0.0     2       4575 197 30
25      4514    0.0     2       4303 183 28
26      4180    0.0     2       3950 201 29
27      4006    0.0     2       3820 154 32
28      3839    0.0     2       3635 176 28
29      3401    0.0     2       3220 150 28 3
30      3242    0.0     3       3080 134 19 9
31      2915    0.0     3       2662 202 33 18
```

```
32      2641    0.0     3       2494 116 18 13
33      2537    0.0     3       2380 118 30 9
34      2287    0.0     3       2147 108 23 9
35      2041    0.0     3       1923 89 18 11
36      1959    0.0     3       1832 100 21 6
37      1865    0.0     3       1738 94 27 6
38      1702    0.0     3       1590 89 14 9
39      1613    0.0     3       1512 73 16 12
40      1389    0.0     3       1311 56 13 9
41      1217    0.0     3       1138 56 12 11
42      1093    0.0     3       1040 38 12 3
43      1004    0.0     3       948 37 12 7
44      914     0.0     3       854 44 6 10
45      862     0.0     3       789 48 14 11
46      845     0.0     3       796 34 5 10
47      718     0.0     3       672 39 4 3
48      714     0.0     3       646 50 14 4
49      690     0.0     3       637 35 13 5
50      592     0.0     3       548 32 7 5
51      515     0.0     3       467 34 11 3
52      474     0.0     3       438 26 4 6
53      470     0.0     3       433 22 9 6
54      373     0.0     3       340 21 5 7
55      377     0.0     3       342 22 6 7
56      319     0.0     3       293 19 6 1
57      330     0.0     3       307 14 6 3
58      305     0.0     3       278 18 4 5
59      281     0.0     3       255 14 10 2
60      288     0.0     3       261 16 9 2
61      258     0.0     3       238 15 3 2
62      215     0.0     3       199 9 6 1
63      239     0.0     3       209 23 3 4
64      214     0.0     3       194 10 8 2
65      173     0.0     3       150 16 4 3
66      166     0.0     3       150 8 4 4
67      142     0.0     3       125 10 6 1
68      130     0.0     3       117 10 1 2
69      107     0.0     3       93 6 6 2
70      93      0.0     3       84 5 3 1
71      80      0.0     3       72 5 1 2
```

```
72      100     0.0     3       86 7 5 2
73      84      0.0     3       70 8 2 4
74      61      0.0     3       54 3 2 2
75      39      0.0     3       33 2 3 1
76      33      0.0     3       30 1 1 1
77      18      0.0     3       15 2 0 1
78      16      0.0     3       16
79      22      0.0     3       16 3 0 3
80      7       0.0     3       6 1
81      8       0.0     3       7 0 1
82      4       0.0     3       4
83      7       0.0     3       7
84      2       0.0     3       1 0 0 1
85      10      0.0     3       6 1 2 1
86      5       0.0     3       4 1
87      3       0.0     3       2 0 0 1
88      1       0.0     3       1
89      2       0.0     3       2
90      3       0.0     3       2 1
91      4       0.0     3       3 0 1
92      3       0.0     3       2 1
93      1       0.0     3       1
95      2       0.0     3       2
96      2       0.0     3       1 1
98      1       0.0     3       0 0 0 1
99      2       0.0     3       0 0 2
101     680     0.0     3       0 597 73 10
```

Trimming

```
Usage:
       PE [-version] [-threads <threads>] [-phred33|-phred64] [-trimlog
<trimLogFile>] [-summary <statsSummaryFile>] [-quiet] [-validatePairs] [-
basein <inputBase> | <inputFile1> <inputFile2>] [-baseout <outputBase> |
<outputFile1P> <outputFile1U> <outputFile2P> <outputFile2U>] <trimmer1>...
```

Resource:
http://www.usadellab.org/cms/?page=trimmomatic
" For paired-end data, two input files are specified, and 4 output files, 2 for the 'paired' output

where both reads survived the processing, and 2 for corresponding 'unpaired' output where a read survived, but the partner read did not."

- LEADING: quality of 3
- TRAILING: quality of 3
- SLIDING WINDOW: window size of 5 and required quality of 15
- MINLENGTH: 35 bases

Be sure to output compressed files and clear out any intermediate files.

- Does file extensions with gz just do this automatically?

Commands:

```
trimmomatic PE -phred33 15_R1_adapters_removed.fastq
15_R2_adapters_removed.fastq 15_R1_adapters_removed_paired.fq.gz
15_R1_adapters_removed_unpaired.fq.gz 15_R2_adapters_removed_paired.fq.gz
15_R2_adapters_removed_unpaired.fq.gz LEADING:3 TRAILING:3 SLIDINGWINDOW:5:15
MINLEN:35

OUTPUT:
TrimmomaticPE: Started with arguments:
 -phred33 15_R1_adapters_removed.fastq 15_R2_adapters_removed.fastq
15_R1_adapters_removed_paired.fq.gz 15_R1_adapters_removed_unpaired.fq.gz
15_R2_adapters_removed_paired.fq.gz 15_R2_adapters_removed_unpaired.fq.gz
LEADING:3 TRAILING:3 SLIDINGWINDOW:5:15 MINLEN:35
Input Read Pairs: 7806403 Both Surviving: 7418387 (95.03%) Forward Only
Surviving: 377369 (4.83%) Reverse Only Surviving: 5675 (0.07%) Dropped: 4972
(0.06%)
TrimmomaticPE: Completed successfully

trimmomatic PE -phred33 17_R1_adapters_removed.fastq
17_R2_adapters_removed.fastq 17_R1_adapters_removed_paired.fq.gz
17_R1_adapters_removed_unpaired.fq.gz 17_R2_adapters_removed_paired.fq.gz
17_R2_adapters_removed_unpaired.fq.gz LEADING:3 TRAILING:3 SLIDINGWINDOW:5:15
MINLEN:35

OUTPUT:
TrimmomaticPE: Started with arguments:
 -phred33 17_R1_adapters_removed.fastq 17_R2_adapters_removed.fastq
17_R1_adapters_removed_paired.fq.gz 17_R1_adapters_removed_unpaired.fq.gz
```

```
17_R2_adapters_removed_paired.fq.gz 17_R2_adapters_removed_unpaired.fq.gz
LEADING:3 TRAILING:3 SLIDINGWINDOW:5:15 MINLEN:35
Input Read Pairs: 11784410 Both Surviving: 11240766 (95.39%) Forward Only
Surviving: 461940 (3.92%) Reverse Only Surviving: 8602 (0.07%) Dropped: 73102
(0.62%)
TrimmomaticPE: Completed successfully
```

Plot trimmed length distributions for R1 and R2, on the same plot
plot_length_dist.py
I guess put the paired and unpaired distributions on the same plot? Should I designate them as paired and unpaired?
Did it, committed it. Considering doing another bit where I plot cumulative distributions rather than the paired and unpaired reads separately.

Part 3

Matplotlib and numpy already installed above.

star

In QAA environment, run:

```
conda install star -c bioconda
conda activate QAA # prev install boots me from environment
conda install bioconda::htseq
```

Hmmm in retrospect, because of the being booted from the environment thing, I may have actually installed matplotlib and numpy in the base environment. Running:

```
conda install matplotlib
```

From there, all numpy packages were installed.

Making some folders, getting some data, unzipping:

```
mkdir gtf_primary_assembly
mkdir lib_17_ALIGNMENT
mkdir lib_15_ALIGNMENT
```

```
mkdir mouse_GENOME_INDEX
cd gtf_primary_assembly
wget https://ftp.ensembl.org/pub/release-
112/fasta/mus_musculus/dna/Mus_musculus.GRCm39.dna_sm.primary_assembly.fa.gz
wget https://ftp.ensembl.org/pub/release-
112/gtf/mus_musculus/Mus_musculus.GRCm39.112.gtf.gz
gunzip Mus_musculus.GRCm39.112.gtf.gz
Mus_musculus.GRCm39.dna_sm.primary_assembly.fa.gz
```

Corrected ps8 and pushed. Copying over code files:

```
cp /projects/bgmp/ghach/bioinfo/Bi621/PS/PS8/ps8-graceHach/src/alignReads.sh .
cp /projects/bgmp/ghach/bioinfo/Bi621/PS/PS8/ps8-graceHach/src/genGen.sh .
cp /projects/bgmp/ghach/bioinfo/Bi621/PS/PS8/ps8-graceHach/src/parse_sam.py .
```

Edited genGen.sh for this project.

```
sbatch genGen.sh
# Checked log files for successful completion
```

Edited alignReads.sh for two runs.
One thing I'm not sure about is what to do with the orphaned reads. For the time being, I'm leaving them out.
Ran super fast.

Created shell script parse_sam.sh.

```
chmod 755 parse_sam.sh
./parse_sam.sh

# RESULTS:
RESULTS for lib_17_ALIGNMENT/Aligned.out.sam
Number of reads mapped: 21532811
Number of reads unmapped: 483272
RESULTS for lib_15_ALIGNMENT/Aligned.out.sam
Number of reads mapped: 14436372
Number of reads unmapped: 207582
```

I think I'll modify this script to help answer #14.

htseq

```
htseq-count [options] <alignment_files> <gtf_file>

# ACTUAL COMMANDS RUN
htseq-count --stranded=yes --counts_output=lib17_strandedyes
lib_17_ALIGNMENT/Aligned.out.sam
gtf_primary_assembly/Mus_musculus.GRCm39.112.gtf
htseq-count --stranded=reverse --counts_output=lib17_strandedrev
lib_17_ALIGNMENT/Aligned.out.sam
gtf_primary_assembly/Mus_musculus.GRCm39.112.gtf
htseq-count --stranded=yes --counts_output=lib15_strandedyes
lib_15_ALIGNMENT/Aligned.out.sam
gtf_primary_assembly/Mus_musculus.GRCm39.112.gtf
htseq-count --stranded=reverse --counts_output=lib15_strandedrev
lib_15_ALIGNMENT/Aligned.out.sam
gtf_primary_assembly/Mus_musculus.GRCm39.112.gtf
# Put into script run_htcount.sh
```

From documentation:
"For `stranded=yes` and paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. For `stranded=reverse`, these rules are reversed"

The Strandedness Question

What does it mean for a library to be stranded?
-> keep track of which strand is the coding strand, and which is the template strand.

It's stranded:

		lib 17 tota;			lib 15 total
lib 17 - reverse		11240766	lib 15 - reverse		7418387
__no_feature	1096110	9.75%	__no_feature	600808	8.10%
__ambiguous	171283	1.52%	__ambiguous	121053	1.63%
__too_low_aQual	16498	0.15%	__too_low_aQual	13832	0.19%
__not_aligned	465461	4.14%	__not_aligned	192825	2.60%
__alignment_not_unique	540050	4.80%	__alignment_not_unique	325272	4.38%
all	2289402	20.37%		1253790	16.90%
lib 17 - yes			lib 15 - yes		
__no_feature	9794540	87.13%	__no_feature	6612851	89.14%
__ambiguous	9671	0.09%	__ambiguous	6232	0.08%
__too_low_aQual	16498	0.15%	__too_low_aQual	13832	0.19%
__not_aligned	465461	4.14%	__not_aligned	192825	2.60%
__alignment_not_unique	540050	4.80%	__alignment_not_unique	325272	4.38%
	10826220	96.31%		7151012	96.40%
Yes didn't seem to work very well			reverse worked, which means that the first read has to be on the opposite strand, and the second read has to be on the main strand		

AAAAAAAA SOMETHING BAD HAPPENED TO THE ENVIRONMENT. I cannot run ./plot_length_dist.sh in QAA environment. I get an incomprehensible error trying to save. Versions of matplotlib and numpy are the same:

```
(QAA) [ghach@n0349 QAA]$ conda list -n QAA numpy
# packages in environment at /gpfs/projects/bgmp/ghach/miniforge3/envs/QAA:
#
# Name                    Version                   Build  Channel
numpy                     2.1.1                    pypi_0    pypi
(QAA) [ghach@n0349 QAA]$ conda list numpy
# packages in environment at /gpfs/projects/bgmp/ghach/miniforge3/envs/QAA:
#
# Name                    Version                   Build  Channel
numpy                     2.1.1                    pypi_0    pypi
(QAA) [ghach@n0349 QAA]$ conda list matplotlib
# packages in environment at /gpfs/projects/bgmp/ghach/miniforge3/envs/QAA:
#
# Name                    Version                   Build  Channel
matplotlib                3.9.2           py312h7900ff3_0    conda-forge
matplotlib-base           3.9.2           py312h854627b_0    conda-forge
(QAA) [ghach@n0349 QAA]$ conda list -n QAA matplotlib
# packages in environment at /gpfs/projects/bgmp/ghach/miniforge3/envs/QAA:
#
# Name                    Version                   Build  Channel
matplotlib                3.9.2           py312h7900ff3_0    conda-forge
matplotlib-base           3.9.2           py312h854627b_0    conda-forge
```