QAA

James Adler

9/1/2021

Part 1 - Read quality score distributions

1.

```
# load fastqc module
module load fastqc/0.11.5

# run fastqc
fastqc /projects/bgmp/shared/2017_sequencing/demultiplexed/31_4F_fox_S22_L008_R1_001.fastq.gz \
    /projects/bgmp/shared/2017_sequencing/demultiplexed/31_4F_fox_S22_L008_R2_001.fastq.gz \
    -o fox \
    -t 8

fastqc /projects/bgmp/shared/2017_sequencing/demultiplexed/23_4A_control_S17_L008_R1_001.fastq.gz \
    /projects/bgmp/shared/2017_sequencing/demultiplexed/23_4A_control_S17_L008_R2_001.fastq.gz \
    -o control \
    -t 8


# copy html output to local folder
scp jadler2@talapas-ln1.uoregon.edu:/projects/bgmp/jadler2/bioinfo/Bi623/QAA/fox/*.html .
scp jadler2@talapas-ln1.uoregon.edu:/projects/bgmp/jadler2/bioinfo/Bi623/QAA/control/*.html .
```

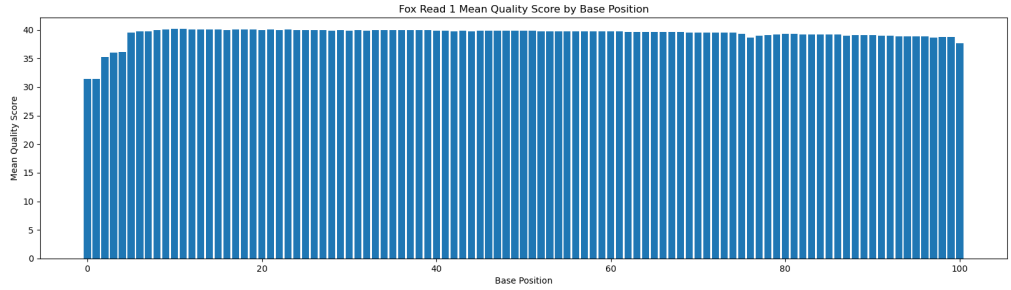
The per-base N quality graphs are consistent with the per-base quality graphs in that base 1 has higher N content relative to the other bases, in alignment with the lower per-base quality score relative to the other bases.

2. Histogram generator

FOX FILES

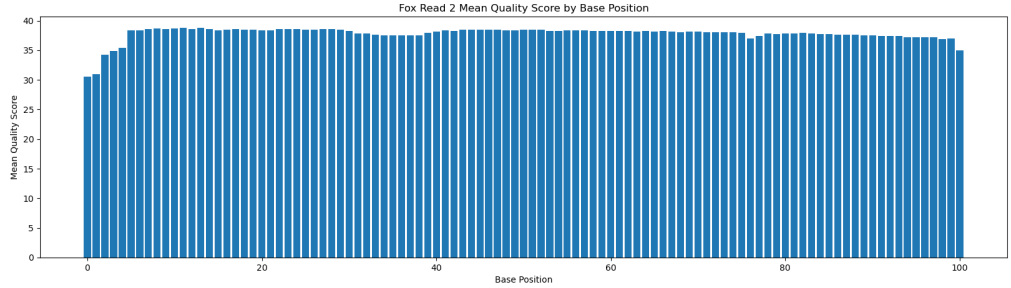
read 1

```
/projects/bgmp/jadler2/bioinfo/Bi623/QAA/python_scripts/histogram_generator.py \
  -f /projects/bgmp/shared/2017_sequencing/demultiplexed/31_4F_fox_S22_L008_R1_001.fastq.gz \
  -l 101 \
  -n 3788343 \
  -o images/31_4F_fox_S22_L008_R1_001.histogram.png \
  -t "Fox Read 1"
```



read 2

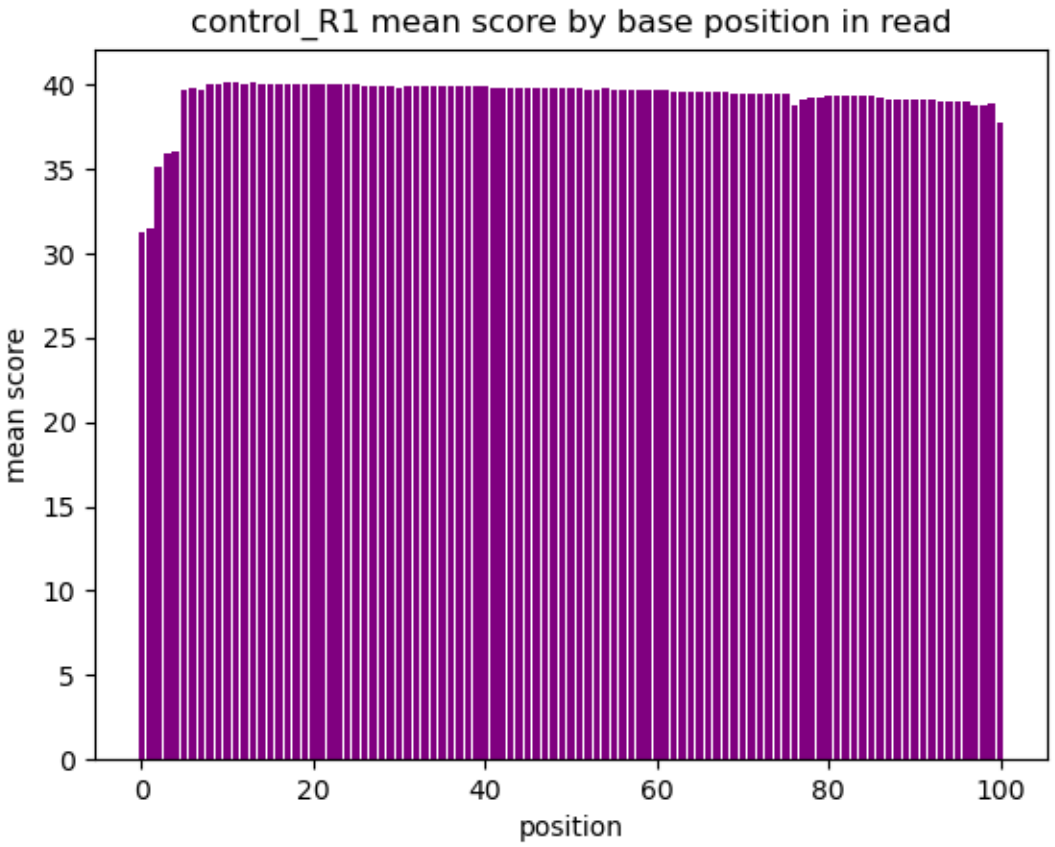
```
/projects/bgmp/jadler2/bioinfo/Bi623/QAA/python_scripts/histogram_generator.py \
  -f /projects/bgmp/shared/2017_sequencing/demultiplexed/31_4F_fox_S22_L008_R2_001.fastq.gz \
  -l 101 \
  -n 3788343 \
  -o images/31_4F_fox_S22_L008_R2_001.histogram.png \
  -t "Fox Read 2"
```



CONTROL FILES

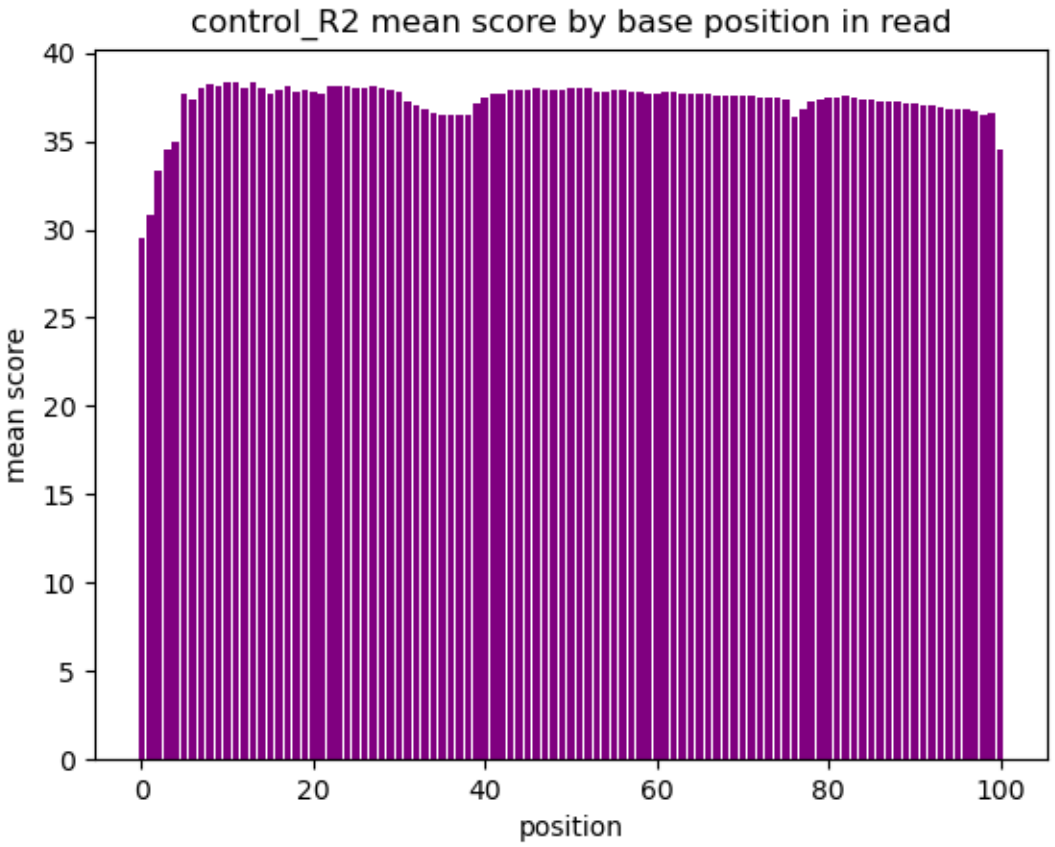
read 1

```
/projects/bgmp/jadler2/bioinfo/Bi623/QAA/python_scripts/histogram_generator.py \
    -f /projects/bgmp/shared/2017_sequencing/demultiplexed/23_4A_control_S17_L008_R1_001.fastq.gz \
    -l 101 \
    -n 44303262 \
    -o images/23_4A_control_S17_L008_R1_001.histogram.png \
    -t "Control Read 1"
```



read 2

```
/projects/bgmp/jadler2/bioinfo/Bi623/QAA/python_scripts/histogram_generator.py \
    -f /projects/bgmp/shared/2017_sequencing/demultiplexed/23_4A_control_S17_L008_R2_001.fastq.gz \
    -l 101 \
    -n 44303262 \
    -o images/23_4A_control_S17_L008_R2_001.histogram.png \
    -t "Control Read 2"
```



Yes, the output and runtimes do differ. The fastqc graphs include box and whiskers to demonstrate the range of values found at each base. The fastqc charts also include green (28-41), yellow (20-28), and red (0-20) areas that correspond to quality level. This is helpful in providing the viewer with a quick reference of quality level at each base.

Fox files The histogram generator runs for the fox files each took about 3 minutes 45 seconds and only produced the one histogram. The fastqc runs can be run with multiple CPUs using the -t flag. Running the two files with -t 8 results in total runtime of 29 seconds and produces substantially more information relative to our histogram generator script.

Control files The histogram generator runs for the control files each took about 40 minutes. Running fastqc on the two controls files with 8 CPUs result sin total runtime of 3 minutes 45 seconds and produces substantially more information relative to our histogram generator script.

3.

Fox files The quality of each of the read files is high. Neither was flagged for poor quality. The mean score is in the green for each of the read files. The 31_4F_fox_S22_L008_R2_001.fastq.gz file does have some whiskers that drop down into the 'yellow' zone (less than 28 mean quality score), but the mean and majority quality scores hover between 34-41.

Control files The quality for each of the read files is high, though R2 does have quite a few whiskers extending into the yellow range and one whisker that extends into the red range. The mean quality score across all bases are all in the green, though the 1st base mean quality is around 30, the lowest mean quality score of any of the four files we processed.

Part 2 - Adaptor trimming comparison

4.

```
# create new conda env
conda create --name QAA python=3.9
conda activate QAA

# install new packages
conda install cutadapt
conda install Trimmomatic
```

version of cutadapt and Trimmomatic

- cutadapt = 3.4
- Trimmomatic = 0.39

5. Adapter trim

fox files

read 1

```
cutadapt -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA \
    -o /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_31_4F_fox_S22_L008_R1_001.fastq.gz \
    /projects/bgmp/shared/2017_sequencing/demultiplexed/31_4F_fox_S22_L008_R1_001.fastq.gz \
    -j 6
```

read 2

```
cutadapt -a AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT \
    -o /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_31_4F_fox_S22_L008_R2_001.fastq.gz \
    /projects/bgmp/shared/2017_sequencing/demultiplexed/31_4F_fox_S22_L008_R2_001.fastq.gz \
    -j 6
```

control files

read 1

```
cutadapt -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA \
  -o /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_23_4A_control_S17_L008_R1_001.fastq.gz \
  /projects/bgmp/shared/2017_sequencing/demultiplexed/23_4A_control_S17_L008_R1_001.fastq.gz \
  -j 6
```

read 2

```
cutadapt -a AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT \
  -o /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_23_4A_control_S17_L008_R2_001.fastq.gz \
  /projects/bgmp/shared/2017_sequencing/demultiplexed/23_4A_control_S17_L008_R2_001.fastq.gz \
  -j 6
```

confirm adapter were removed

```
zcat trimmed_31_4F_fox_S22_L008_R1_001.fastq.gz | grep "AGATCGGAAGAGCACACGTCTGAACTCCAGTCA"

zcat trimmed_31_4F_fox_S22_L008_R2_001.fastq.gz | grep "AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT"

zcat trimmed_23_4A_control_S17_L008_R1_001.fastq.gz | grep "AGATCGGAAGAGCACACGTCTGAACTCCAGTCA"

zcat trimmed_23_4A_control_S17_L008_R2_001.fastq.gz | grep "AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT"
```

- all returns were empty, confirming that none of the reads present in each of the files contain the adapter sequence

6. Trimmomatic runs

fox files

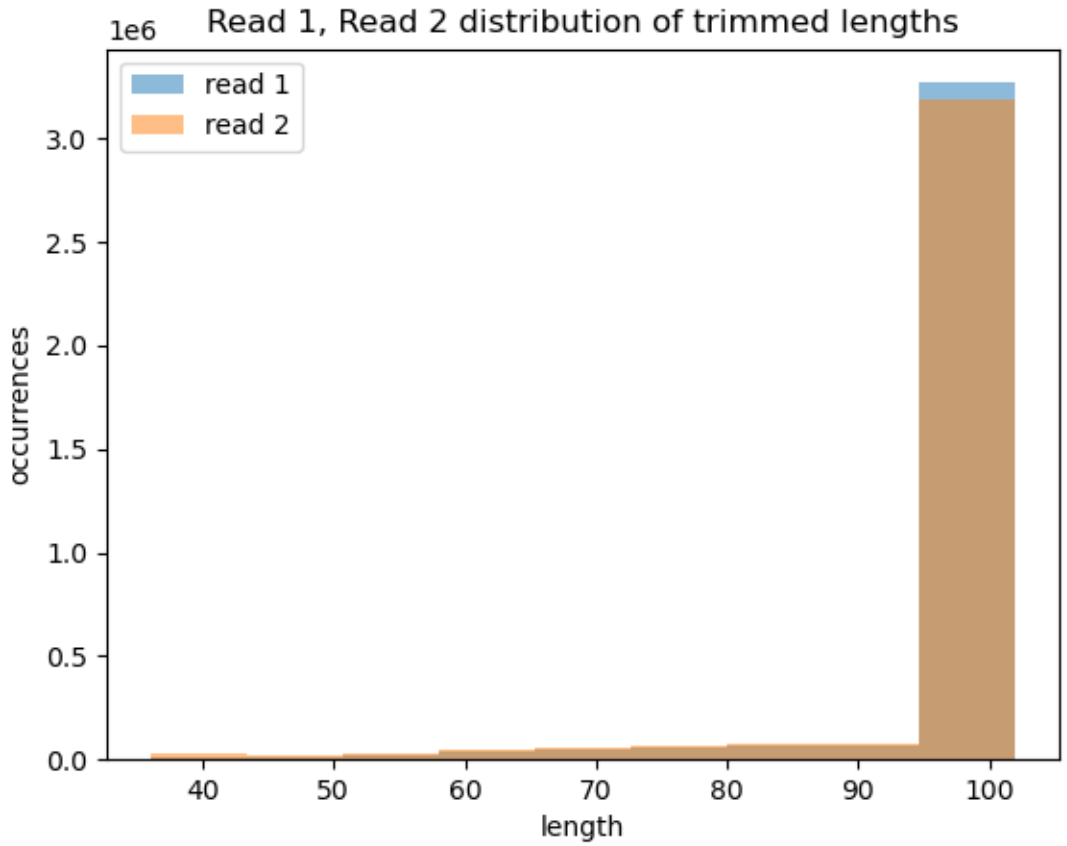
```
java -jar /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Trimmomatic-0.39/trimmomatic-0.39.jar PE \
  -threads 8 /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_31_4F_fox_S22_L008_R1_001.fastq.gz \
  /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_31_4F_fox_S22_L008_R2_001.fastq.gz \
  -baseout /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Trim_Reads/quality_trimmed_31_4F_fox_S22_L008_001.fa
  LEADING:3 \
  TRAILING:3 \
  SLIDINGWINDOW:5:15 \
  MINLEN:35
```

control files

```
java -jar /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Trimmomatic-0.39/trimmomatic-0.39.jar PE \
  -threads 8 /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_23_4A_control_S17_L008_R1_001.fastq.gz \
  /projects/bgmp/jadler2/bioinfo/Bi623/QAA/trimmed_23_4A_control_S17_L008_R1_001.fastq.gz \
  -baseout /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Trim_Reads/quality_trimmed_23_4A_control_S17_L008_R
  LEADING:3 \
  TRAILING:3 \
  SLIDINGWINDOW:5:15 \
  MINLEN:35
```

7.

```
./dual_histogram.py
        -i1 Trim_Reads/quality_trimmed_31_4F_fox_S22_L008_001_1P.fastq.gz
        -i2 Trim_Reads/quality_trimmed_31_4F_fox_S22_L008_001_2P.fastq.gz
        -o trimmed_lengths.histogram.png
        -t "Read 1, Read 2 distribution of trimmed lengths
```



Part 3 - Alignment and strand-specificity

8.

```
conda install star -c bioconda
conda install numpy
conda install pysam
conda install matplotlib
pip install HTSeq
```

9.

```
# wget files
wget http://ftp.ensembl.org/pub/release-104/fasta/mus_musculus/dna/Mus_musculus.GRCm39.dna.primary_asse
wget http://ftp.ensembl.org/pub/release-104/gtf/mus_musculus/Mus_musculus.GRCm39.104.gtf.gz

# generate mouse db
STAR --runThreadN 8
  --runMode genomeGenerate
  --genomeDir /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Mus_musculus.GRCm39.dna.ens.104.STAR_2.7.9a
  --genomeFastaFiles /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Mus_musculus.GRCm39.dna.primary_assembly.
  --sjdbGTFfile /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Mus_musculus.GRCm39.104.gtf
```

```
# align trimmed reads
STAR --runThreadN 8
  --runMode alignReads
  --outFilterMultimapNmax 3
  --outSAMunmapped Within KeepPairs
  --alignIntronMax 1000000
  --alignMatesGapMax 1000000
  --readFilesCommand zcat
  --readFilesIn /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Trim_Reads/quality_trimmed_31_4F_fox_S22_L008_
  /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Trim_Reads/quality_trimmed_31_4F_fox_S22_L008_001_2P.fastq.g
  --genomeDir /projects/bgmp/jadler2/bioinfo/Bi623/QAA/Mus_musculus.GRCm39.dna.ens.104.STAR_2.7.9a
  --outFileNamePrefix ./align/
```

10.

```
# determine mapped vs unmapped
/projects/bgmp/jadler2/bioinfo/Bi621/PS/ps8-adler-sudo/sam_filter.py
  -f /projects/bgmp/jadler2/bioinfo/Bi623/QAA/align/Aligned.out.sam
  -o /projects/bgmp/jadler2/bioinfo/Bi623/QAA/map_v_unmap.txt
```

11.