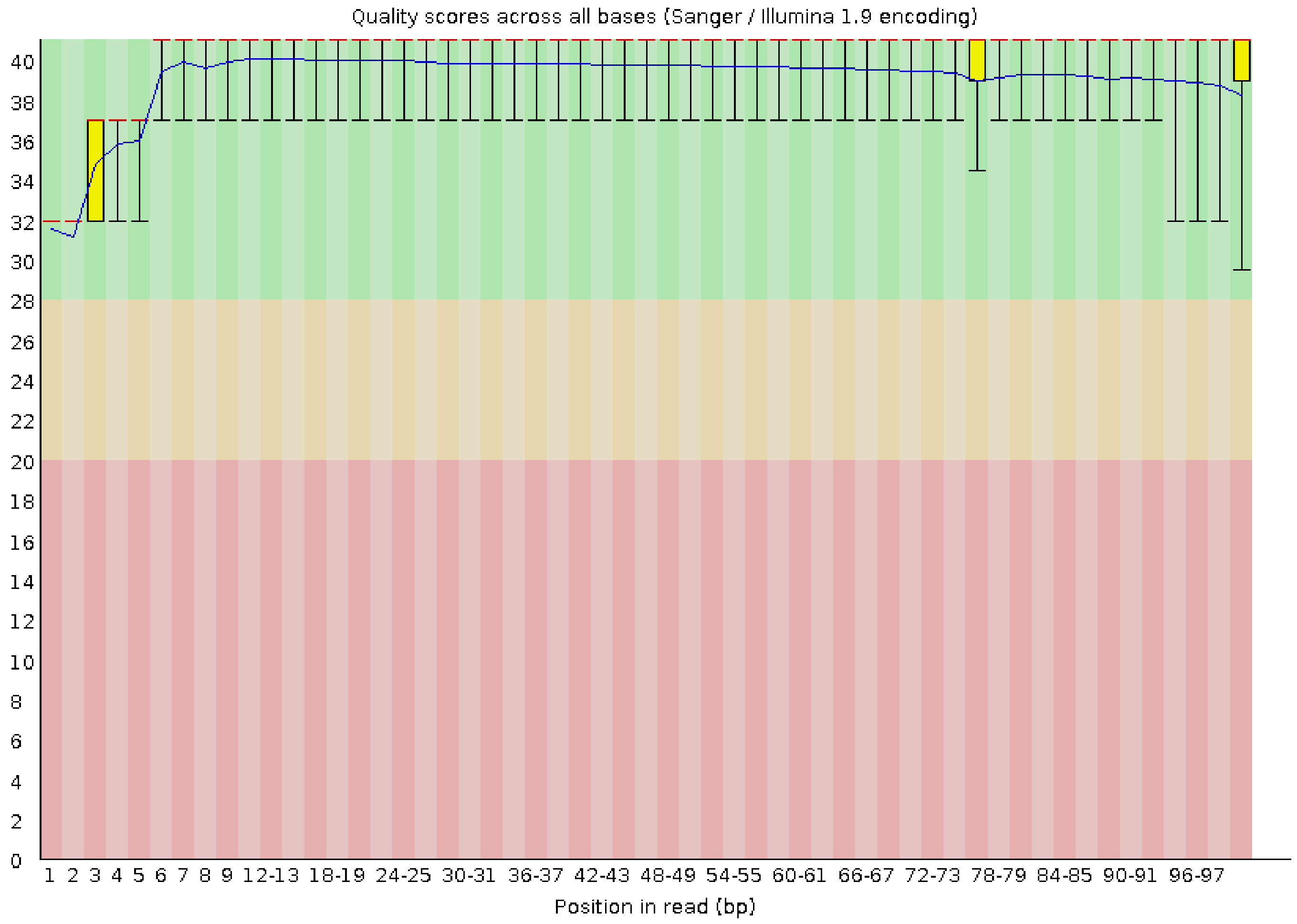
RNA-seq Quality Assessment Assignment - Bi 623 (Summer 2021)

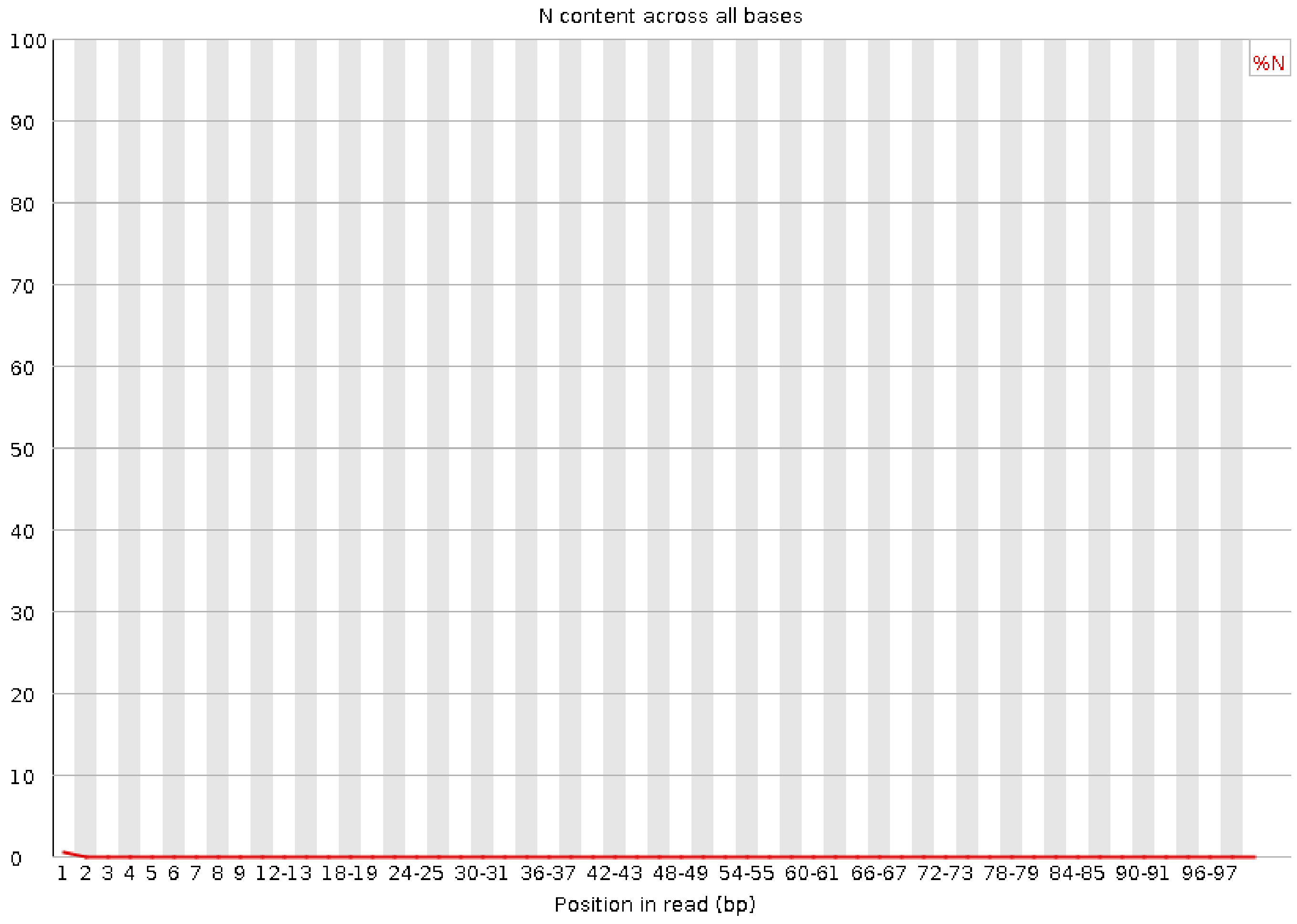
Data:

7_2E_fox_S6_L008
19_3F_fox_S14_L008

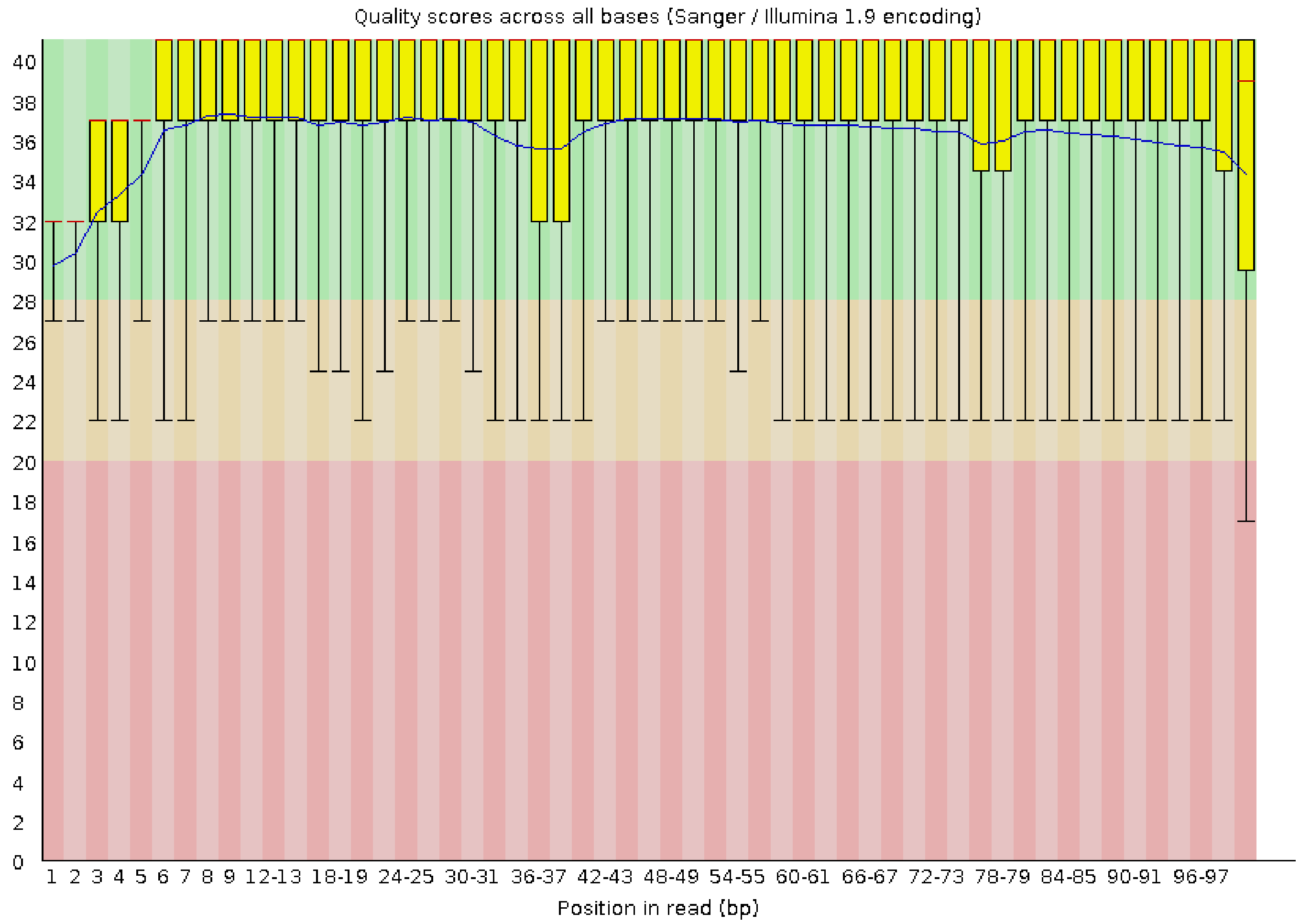
Part 1 – Read quality score distributions

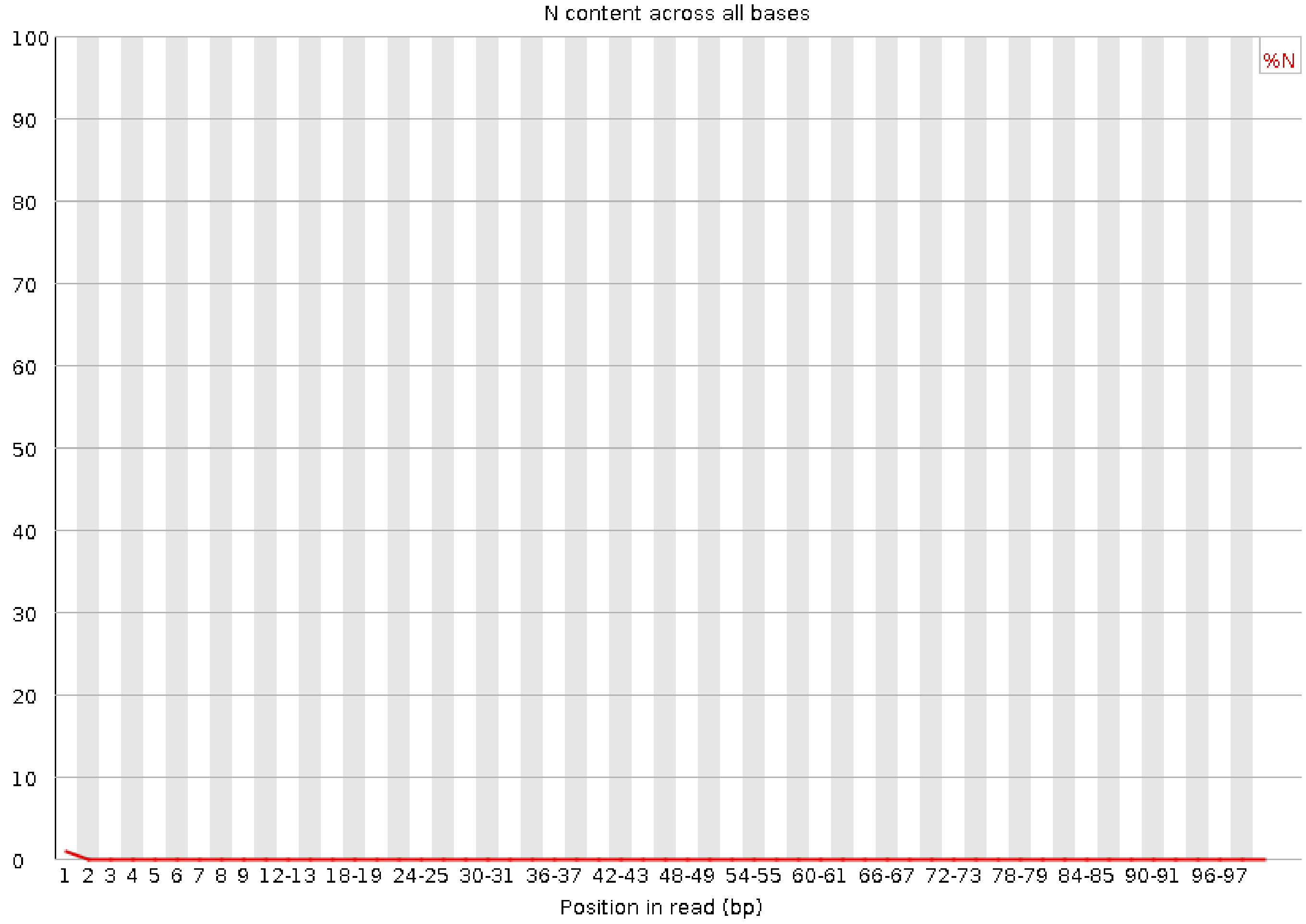
7_2E_Fox Read 1 Graphs


Quality scores across all bases (Sanger / Illumina 1.9 encoding)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)

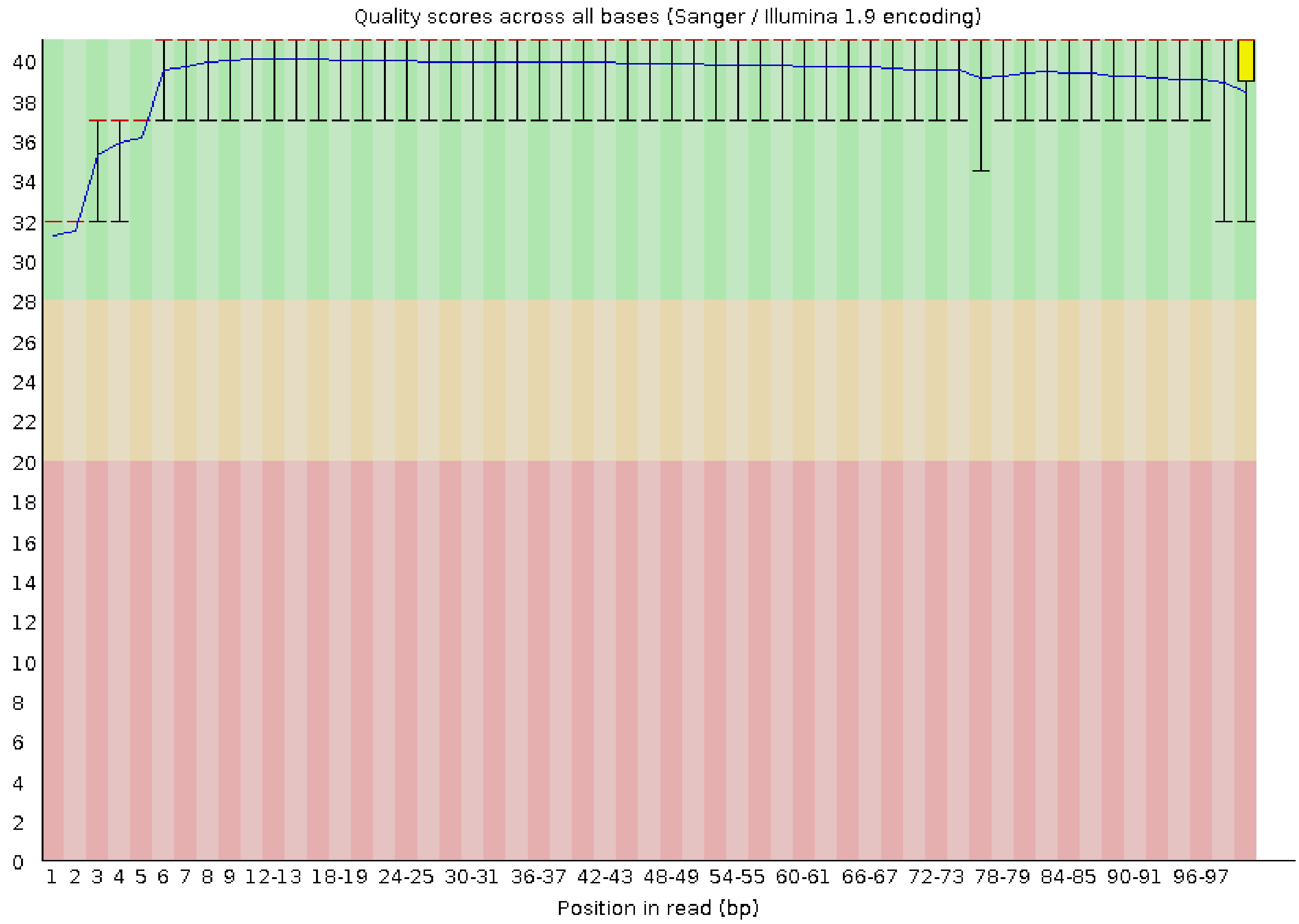

N content across all bases
%N
0
10
20
30
40
50
60
70
80
90
100
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)

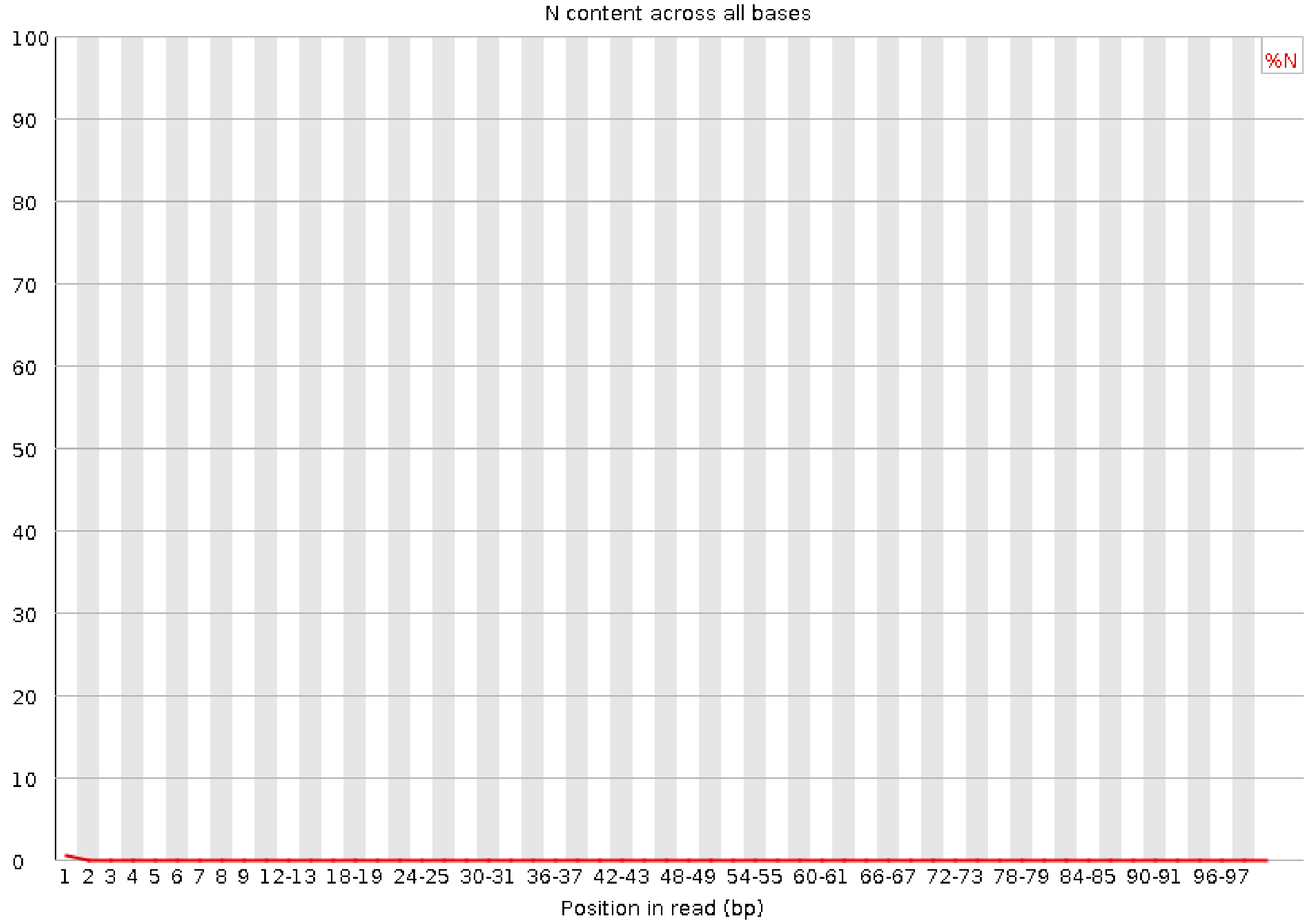
7_2E_Fox Read 2 Graphs


Quality scores across all bases (Sanger / Illumina 1.9 encoding)
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)

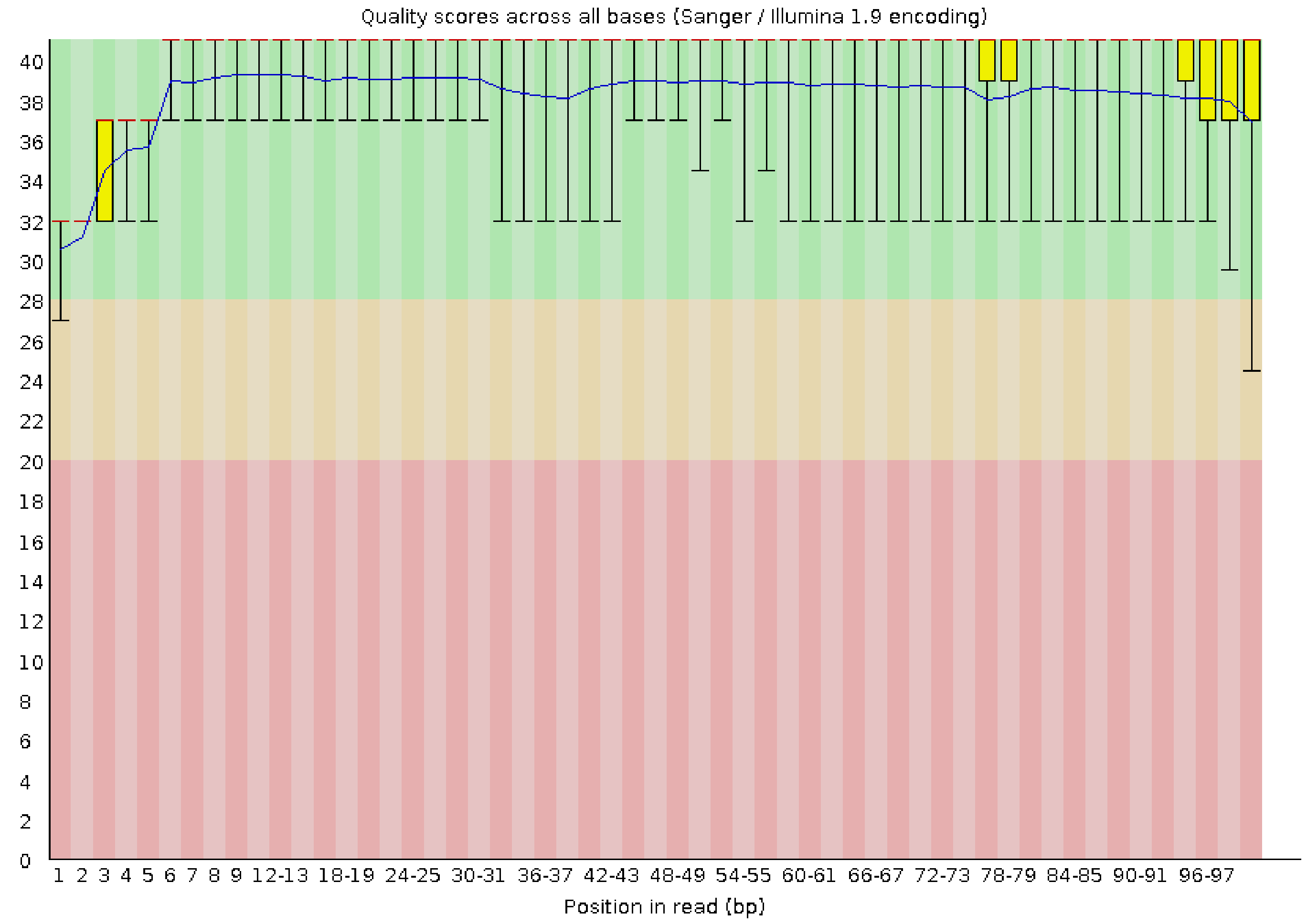

N content across all bases
100
90
80
70
60
50
40
30
20
10
0
%N
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)

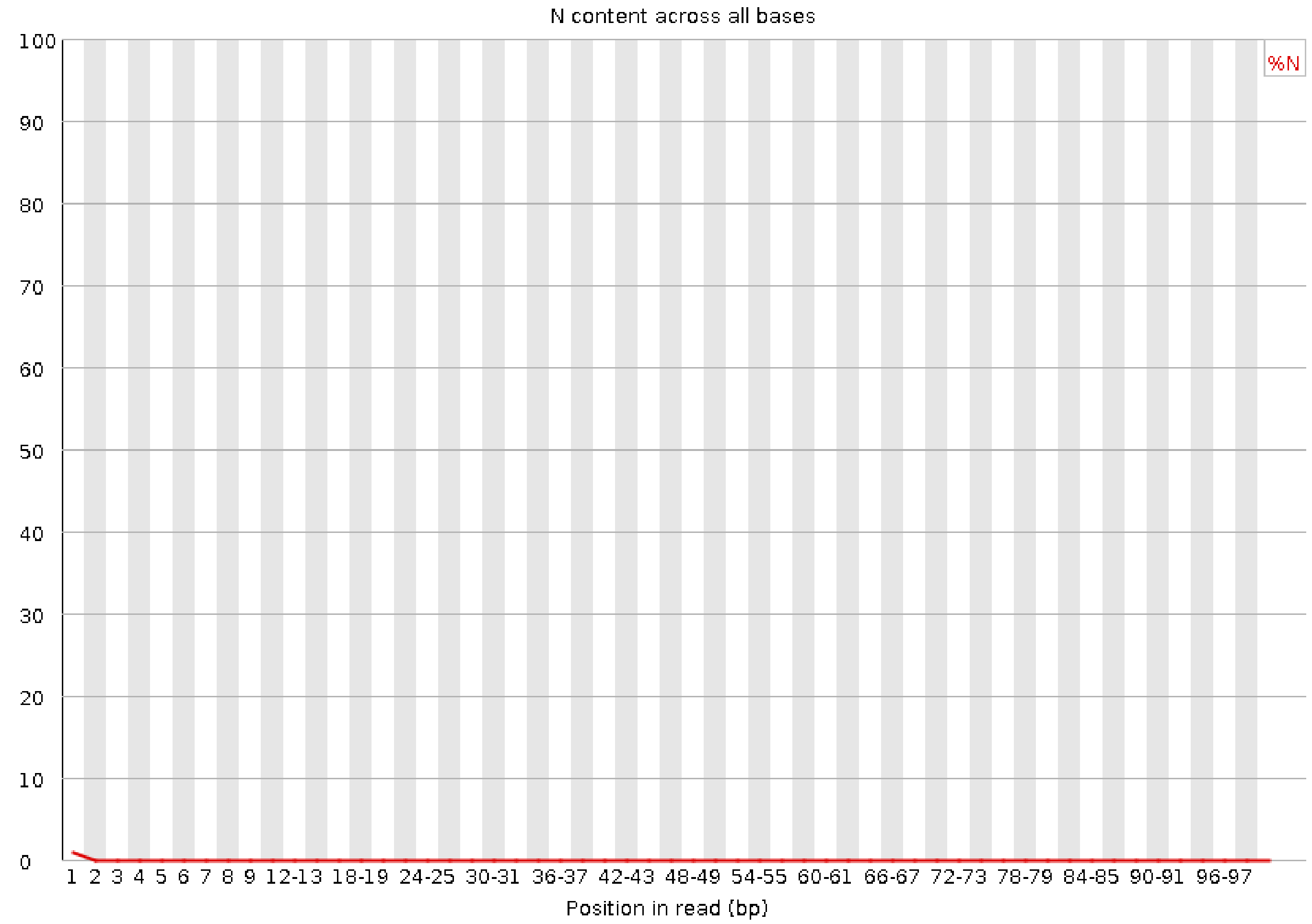
19_3F_Fox Read 1 Graphs


Quality scores across all bases (Sanger / Illumina 1.9 encoding)
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)


N content across all bases
100
90
80
70
60
50
40
30
20
10
0
%N
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)

19_3F_Fox Read 2 Graphs


Quality scores across all bases (Sanger / Illumina 1.9 encoding)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)


N content across all bases
%N
0
10
20
30
40
50
60
70
80
90
100
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97
Position in read (bp)

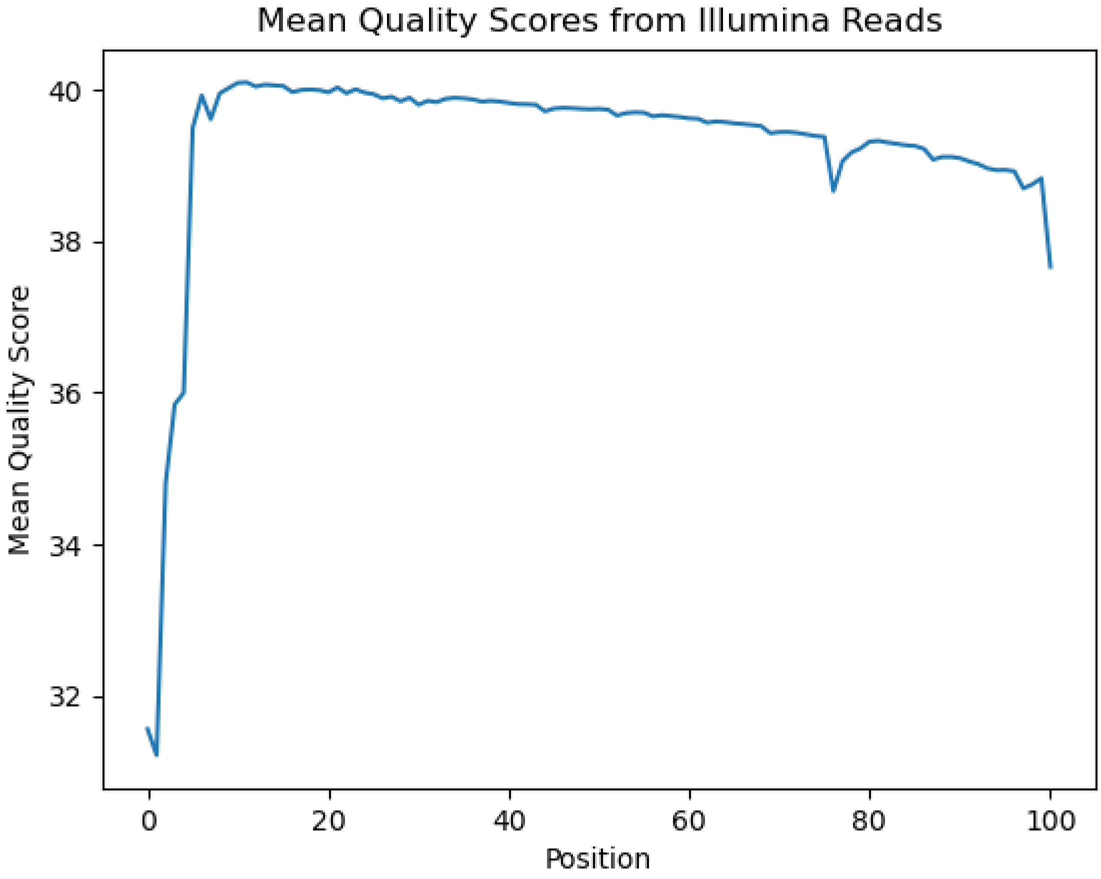
Answers

Are the per-base N plots consistent with the quality score plots?

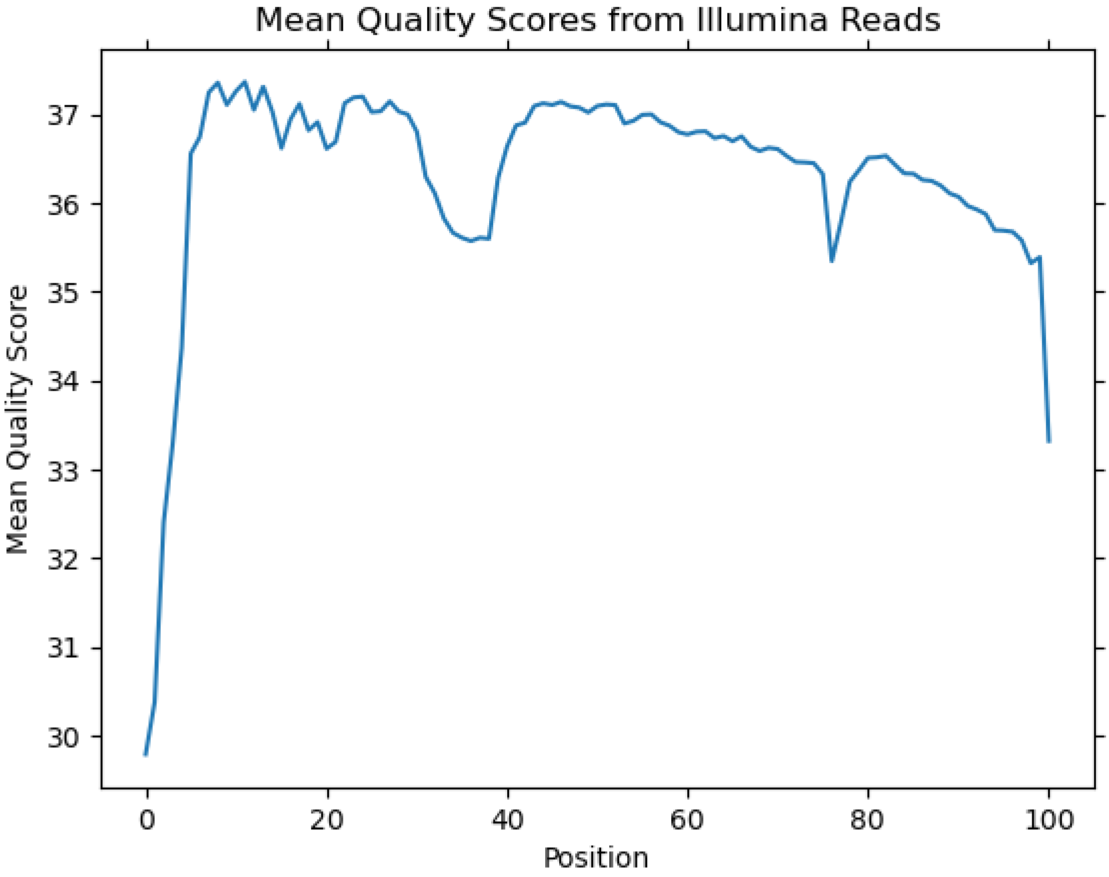
```
The per-base N plots appear to be mostly consistent with the quality score
plots. In all of the above plots we see a rise in the numebr of N's over
the first few reads in the N content graphs. This is mirrored in the lower
average quality score over these same reads in the quality score graphs.
```

Personal Quality Control Graphs

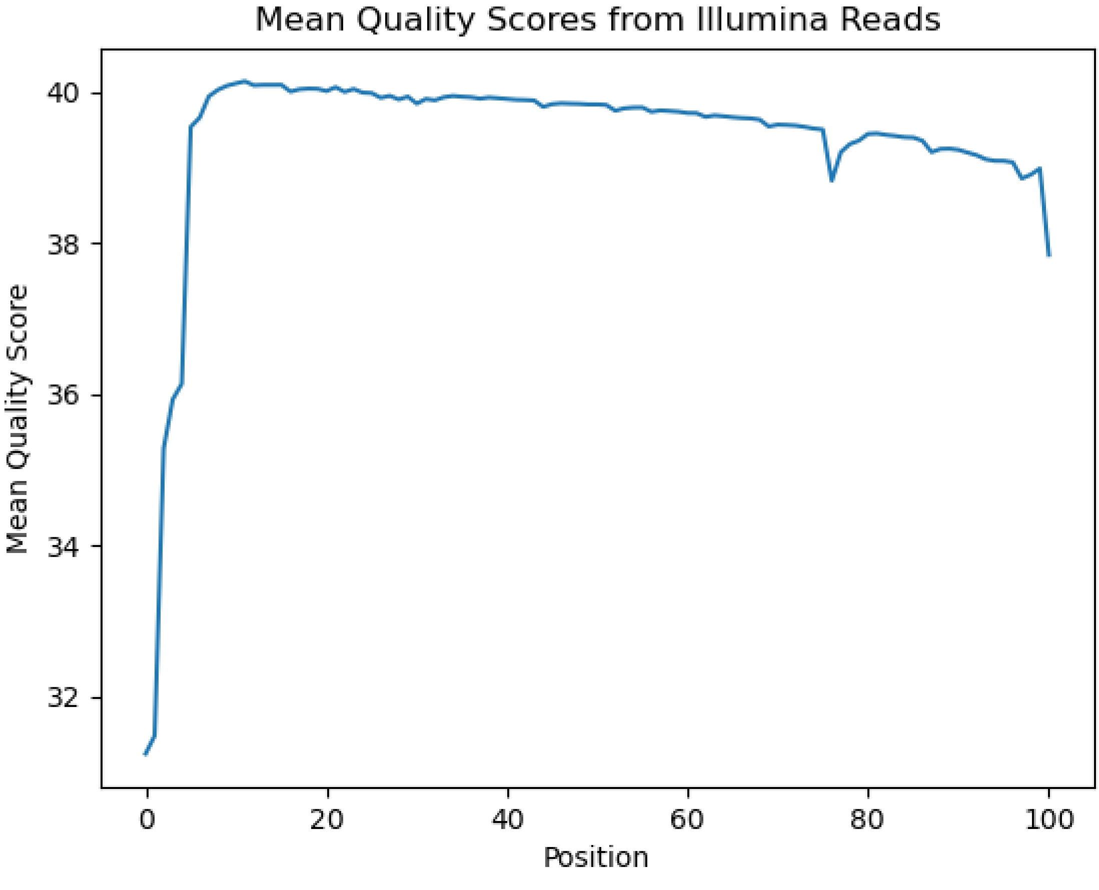
7_2E_Fox Read 1 Graphs



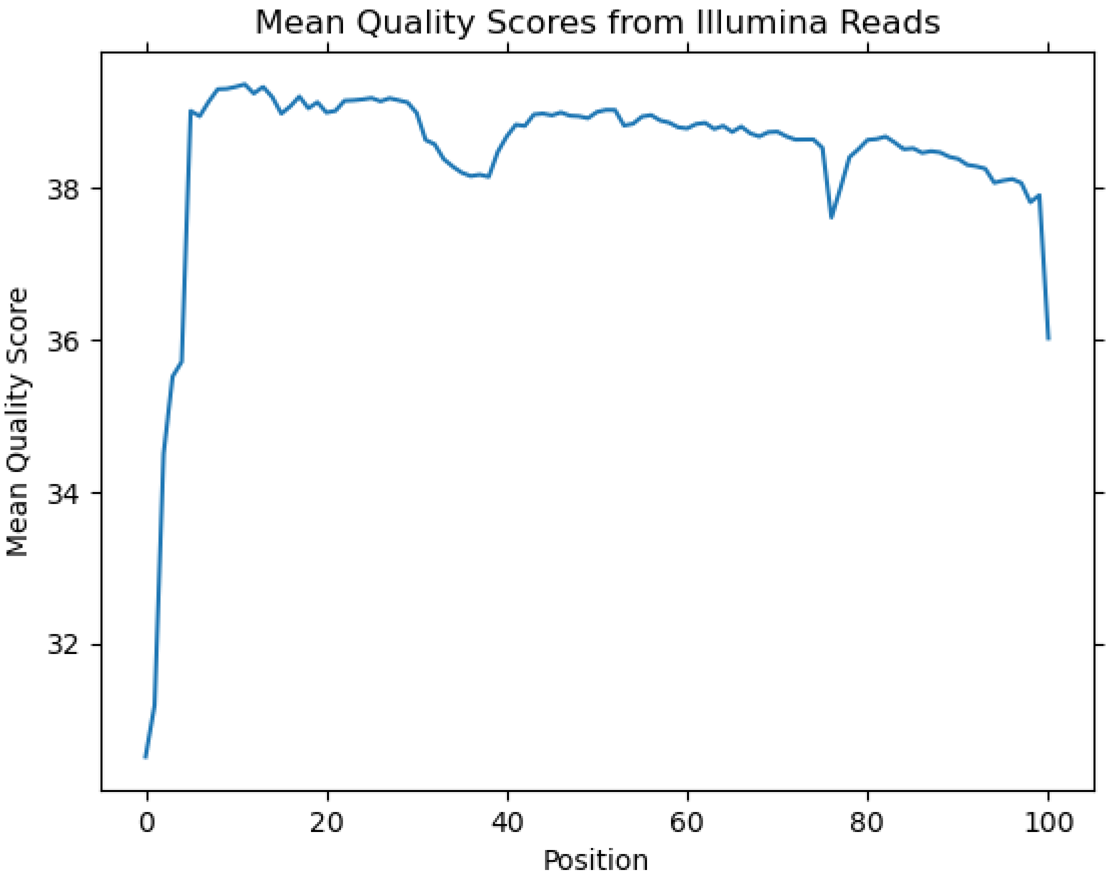
7_2E_Fox Read 2 Graphs



19_3F_Fox Read 1 Graph



19_3F_Fox Read 2 Graph



Answers

Describe how the FastQC quality score distribution plots compare to your own. If different, propose an explanation. Also, does the runtime differ? If so, why?

```
The quality plots are fairly similar to my own. Across all of the data both
graphing methods show a clear decrease in quality scores in the early
positions that quickly ramps after reading begins. In addition, both
methods show a lower average data quality on 7_2E's 2nd read. Overall, I
would say that these graphs mostly mirror each other, however the fastqc
graphs present more information in a better way even if the core
information is the same. Also, the runtime was signficantly shorter for
FastQC as compared to my own algorithm. If I had to guess, it would mostly
be due to the inefficency of looping thorugh every line individually
without indexing in my algorithm as compared to the FastQC method.
```

Part 2 – Adaptor trimming comparison

What proportion of reads (both forward and reverse) were trimmed?

```
 7_2E read 1:
 Total reads processed:               5,278,425
 Reads with adapters:                   173,473 (3.3%)
 3.3% of total reads were trimmed

 7_2E read 2:
 Total reads processed:               5,278,425
 Reads with adapters:                   212,512 (4.0%)
 4.0% of total reads were trimmed

 19_3F read 1:
 Total reads processed:              16,348,255
 Reads with adapters:                   546,623 (3.3%)
 3.3% of total reads were trimmed

 19_3F read 2:
 Total reads processed:              16,348,255
 Reads with adapters:                   676,564 (4.1%)
 4.1% of total reads were trimmed
```

Sanity check: Use your Unix skills to search for the adapter sequences in your datasets and confirm the expected sequence orientations. Report the commands you used, the reasoning behind them, and how you confirmed the adapter sequences.

```
A quick sanity check after googling the adaters that were used in the kit
that this experiment with done with is shown below. The easiest way to see
if these adatpers show up in the data is a quick a grep. This will hilight
where the sequence is found with in the file and will let us look at every
line where it is found. We would expect that these adapters should appear
after the insert is sequenced so we would not expect many instances of it
at the start of the line and instead majority of them at the end. While
this method doesn't allow us to  check if the adapter is partially present
in our reads it does give us a good indication that is is present in
sufficent quantites in our reads.


Code examples:
This will visually show where the adapter is found in this data set.

zcat /projects/bgmp/shared/2017_sequencing/demultiplexed/19_3F_fox_S14_L008_R1_001.fastq.gz | grep 'AGATCGGAAGAGCACACGTCTGAACTCCAGTCA'

This will tell us how much of the adater was found at the start of a read.

zcat /projects/bgmp/shared/2017_sequencing/demultiplexed/19_3F_fox_S14_L008_R1_001.fastq.gz | grep '^AGATCGGAAGAGCACACGTCTGAACTCCAGTCA' | wc -l
```

Plot the trimmed read length distributions for both R1 and R2 reads (on the same plot). You can produce 2 different plots for your 2 different RNA-seq samples. There are a number of ways you could possibly do this. One useful thing your plot should show, for example, is whether R1s are trimmed more extensively than R2s, or vice versa.

Comment on whether you expect R1s and R2s to be adapter-trimmed at different rates.

```
I would expect R2 to be trimmed more extensively tahn R1.
```

Part 3 – Alignment and strand-specificity

Mouse Genome Used

```
Genome name: Mus_musculus.GRCm39.dna.primary_assembly.fa

GTF name: Mus_musculus.GRCm39.104.gtf
```

Count reads that map to features using htseq-count. You should run htseq-count twice: once with --stranded=yes and again with --stranded=no. Use default parameters otherwise.

Demonstrate convincingly whether or not the data are from “strand-specific” RNA-Seq libraries. Include any comands/scripts used. Briefly describe your evidence, using quantitative statements (e.g. "I propose that these data are/are not strand-specific, because X% of the reads are y, as opposed to z.").

Hint - recall ICA4 from Bi621.

To turn in your work for this assignment: Upload your Talapas batch script/code, FastQC plots, mapped/unmapped read counts, counts files generated from htseq-count, answers to questions, and any additional plots/code to github. You should create at most 2 files for submission (R markdown and the rendered pdf file) containing all these elements. The three parts of the assignment should be clearly labeled. Be sure to title and write a figure legend for each image/graph/table you present.