QAA Report

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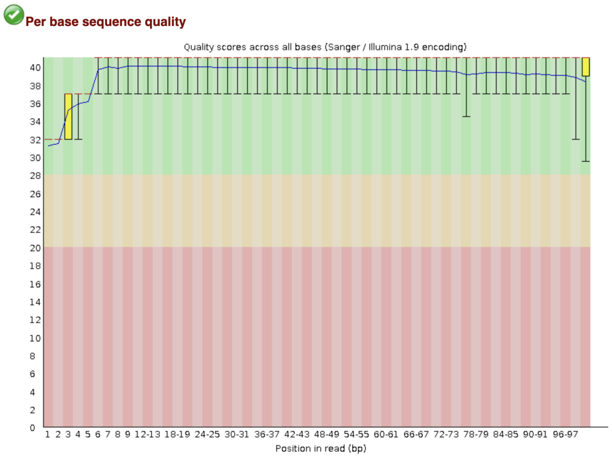
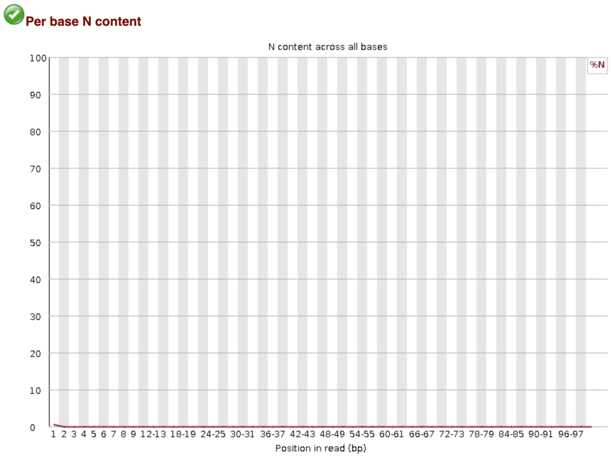
Part 1

FastQC Results Interpretation

The per-base N content plots appear to be consistent with the quality score plots. There is a small uptick in N content at the beginning of sequences and that is reflected by the initial lower quality of sequences. The plots are included below.

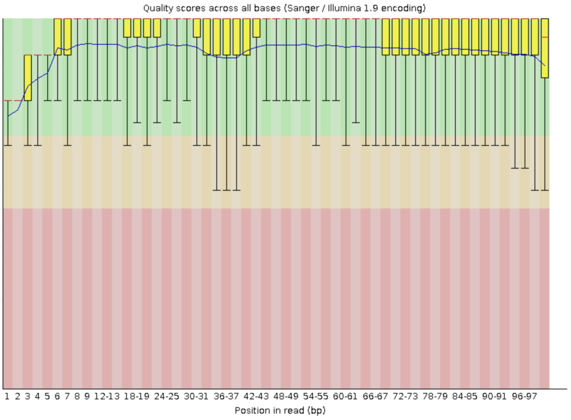
29_4E_fox R1



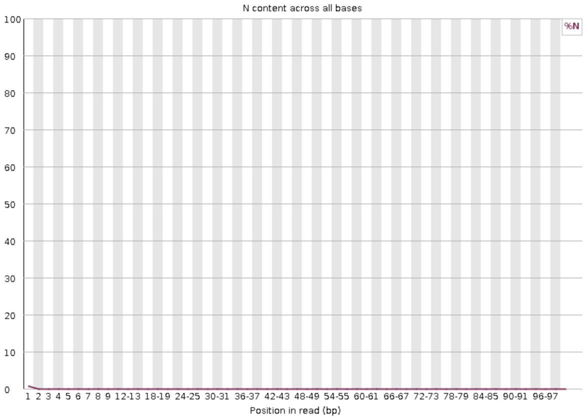


29__4E__fox R2

✓ Per base sequence quality

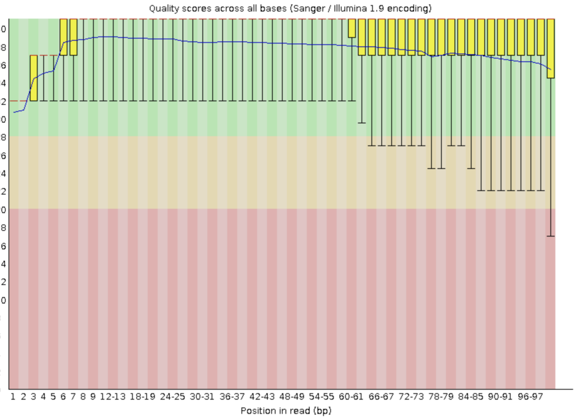


✓ Per base N content

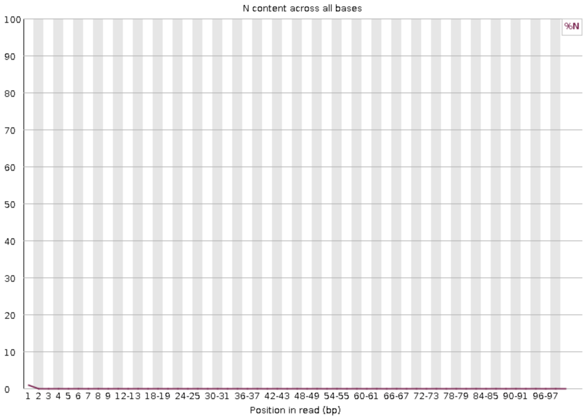


Undetermined R1

✓ Per base sequence quality

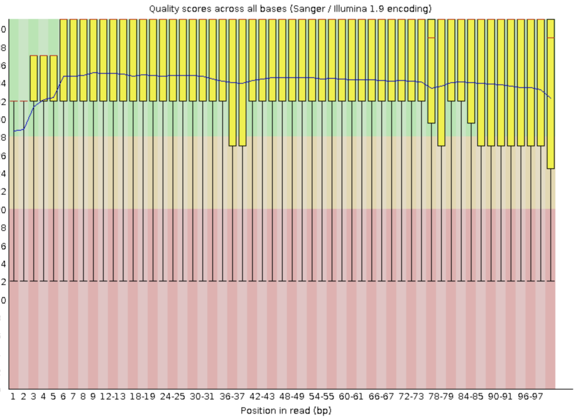


✓ Per base N content

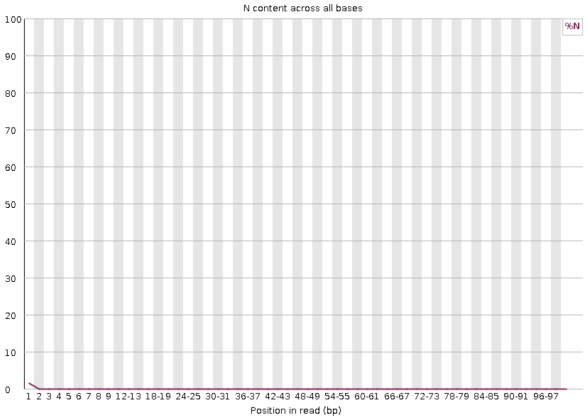


Undetermined R2

✓ Per base sequence quality



✓ Per base N content



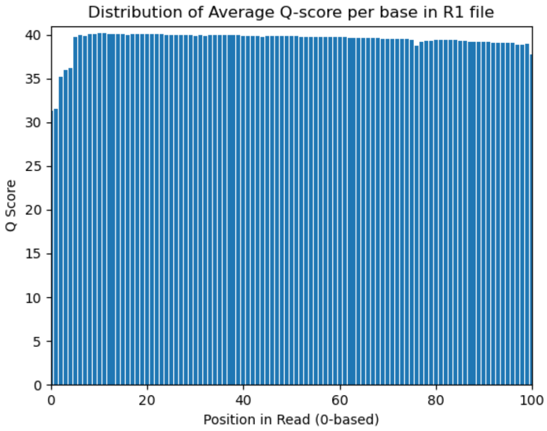
FastQC Plots vs. Demux Plots

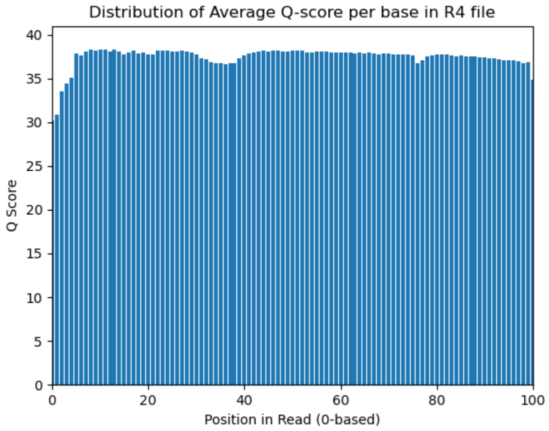
The quality score plots appear to be very similar. Each pair shows nearly (if not) identical scores per base position. There is a difference only in width of bins on my demux plots, which initially made it appear as though my plots were different from FastQC's.

Run time and Mem/CPU usage was rather different, this is to be expected given FastQC is an extremely well established and optimized tool for bioinfomatic analyses. Comparing my personal code to that of such a tool is a bit unfair. I'm confident I could produce better run-times and lower CPU+Memory usage from my code if I was given the opportunity to develop the code from 2010 until now, as FastQC has been.

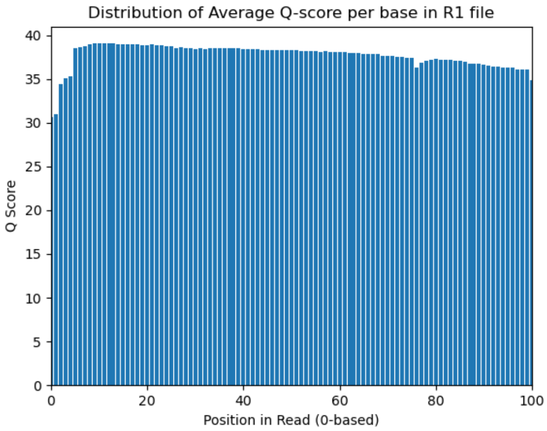
Demux plots included below.

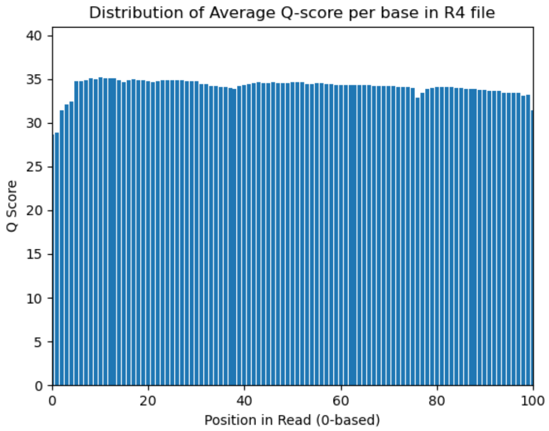
29_4E_fox





Undetermined





Data Quality Analysis

Assessing the entire FastQC report generated for each sample, the 29_4E_fox library appears to be adequate for subsequent analysis. In all four reports, there were 0 reads identified as low quality. The Undetermined library may not be a great choice for further analysis due to high levels of sequence duplication, 20% or so higher than the duplication levels observed in the 29_4E_fox library. Duplication is something that can be dealt with computationally, but generally losing more than half of the library when doing so, isn't a great sign.

Part 2

Trimmed Read %

11.62% reads were trimmed from the Undetermined Library

5.29% reads were trimmed from the 29_4E_fox library

Undetermined Library Trimming Statistics

```
Input Read Pairs:        14760166
Both Surviving:          12160071 (82.38%)
Forward Only Surviving: 2511252 (17.01%)
Reverse Only Surviving: 31174 (0.21%)
Dropped:                 57669 (0.39%)
```

29_4E_fox Library Trimming Statistics

```
Input Read Pairs:        4827433
Both Surviving:          4571904 (94.71%)
Forward Only Surviving: 247896 (5.14%)
Reverse Only Surviving: 3367 (0.07%)
Dropped:                 4266 (0.09%)
```

Sanity Check

Adapter sequences were confirmed by investigating the metadata and thusly the library prep. Although a Kapa kit is utilized, it is not specifically stated what adapter sequences were utilized. The FastQC reports indicate that Illumina Universal Adapters were dectected so I then looked for those and didn't find much. Spoke with Leslie and was directed to an illumina page that had the adapter sequences provided. I think the original site I had found was deprecated and moved to the link that Leslie provided. The adapters are present and identified as TruSeq.

```
zcat <file> | grep '<adapter sequence>' | wc -l
```

This command outputs the count of sequences that contain the adapter sequence.

```
Undetermined R1 adapter sequence count:   30,808
Undetermined R2 adapter sequence count:   34,088
29_4E_fox R1 adapter sequence count:      39,701
29_4E_fox R2 adapter sequence count:      39,929
```

```
zcat <file> | awk -v s="<adapter sequence>" ’i=index($0, s) {print i}’ |\
 awk ’{position+=$0} END {print position/NR}’‘
```

This command outputs the average position of the adapter sequence in the input fastQ file. Awk is first used to identify the position where the adapter appears in each read. This is then piped to another awk that provides an average of the supplied positions. The adapter sequence is 33 characters long, so there should be an average position near/around 68, assuming adapters exist mostly on the 3’ end of reads.

```
Undetermined R1 average adapter position:   57.8274
Undetermined R2 average adapter position:   57.2016
29_4E_fox R1 average adapter position:      60.0634
29_4E_fox R2 average adapter position:      60.0374
```

For both files in the either library, the adapters appear more on the 3’ end of the reads. This is a good sign.

Trimmed Read Length Plots

R1 & R2 files should be trimmed at similar rates, I do expect to see more trimming on Undetermined R2 as its adapter is present more often than R1’s adapter is in R1. This could be due to random chance, and the adapter sequence appearing naturally in other places in the reads, but the average location of the adapter is nearly identical to the average observed for the R1 adapter.

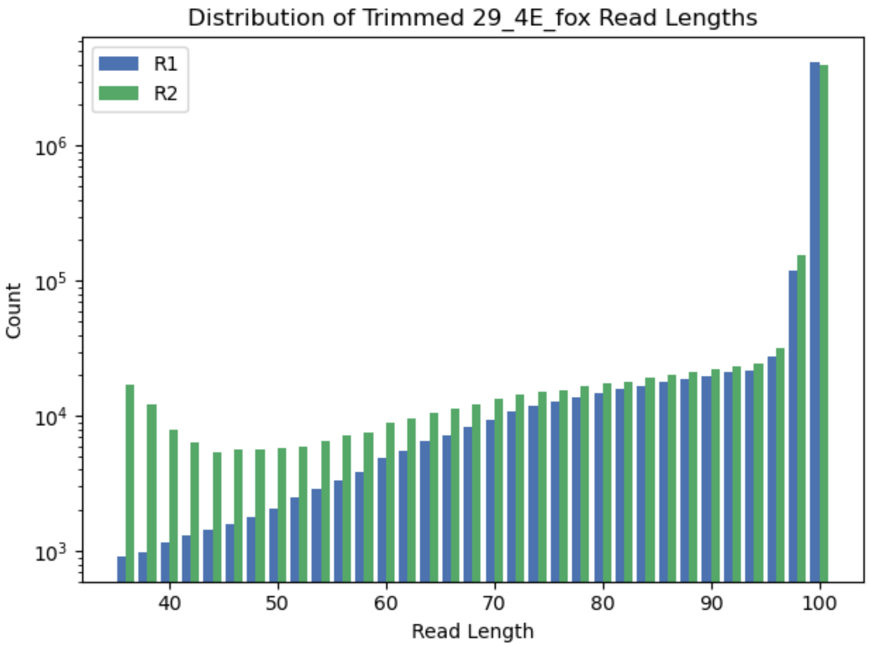


Figure 1: Trimmed Read Lengths of the 29_4E_fox Library

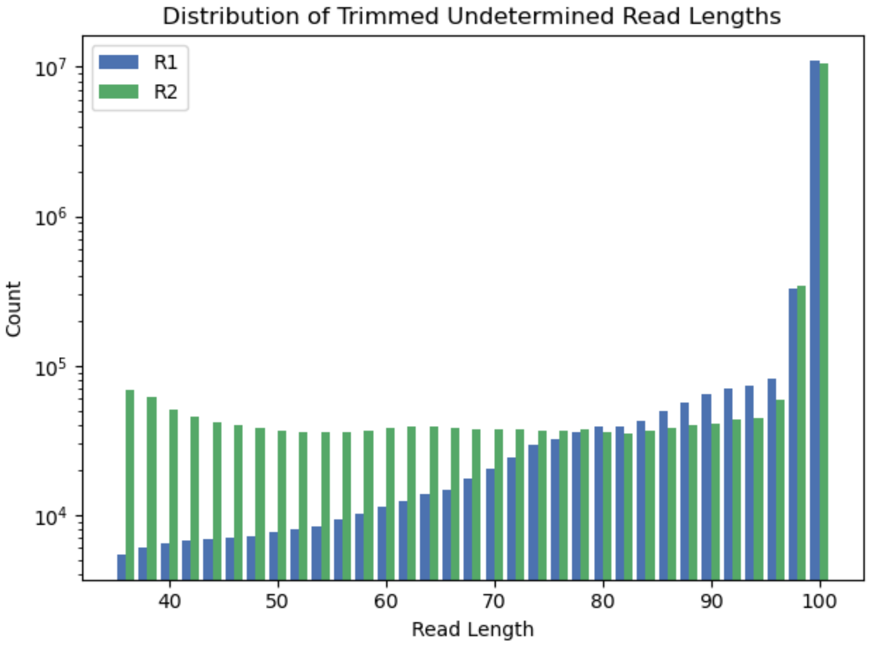
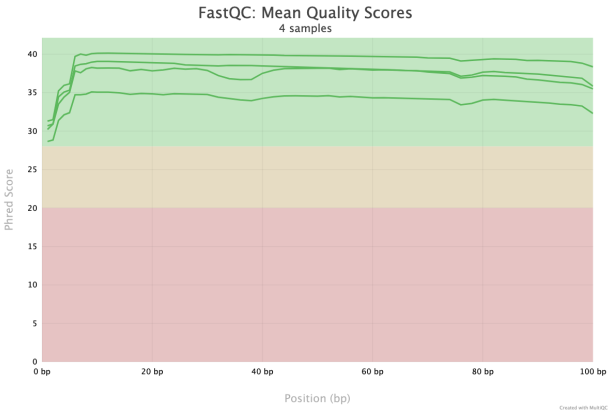


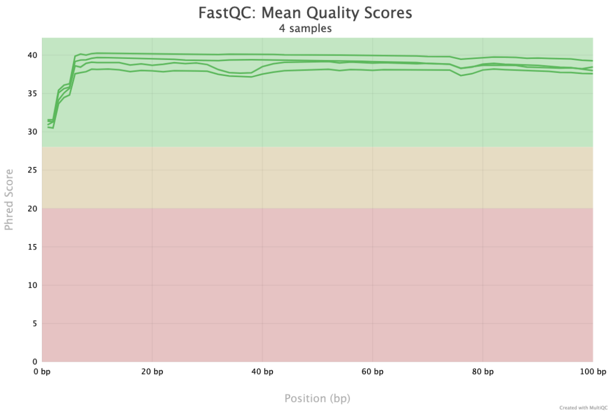
Figure 2: Trimmed Read Lengths of the Undetermined Library

Trimmed FastQC

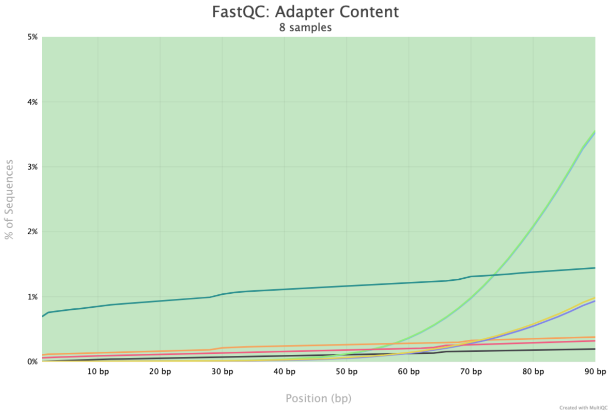
Went ahead and ran multiQC on raw and trimmed fastQC files for easier summary of statistics. The trimmed datasets have an modestly increased sequence quality, likely due to the reduced N content (both sets of plots included below). The trimmed dataset also has a drastically decreased number of adapter sequences, which is to be expected after trimming. Due to the adapters being trimmed, there is also an uptick in quality at the ends of reads.

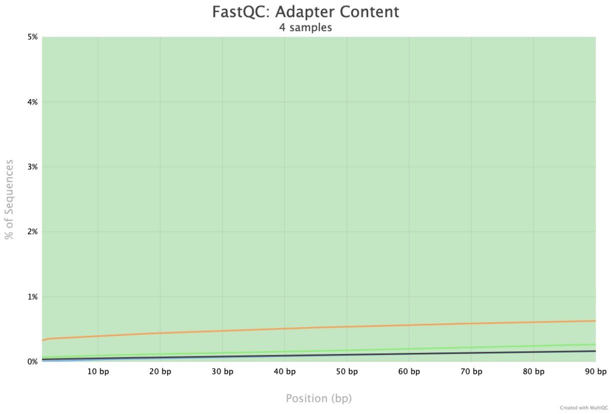
Raw Vs. Trimmed Per Base Sequence Quality



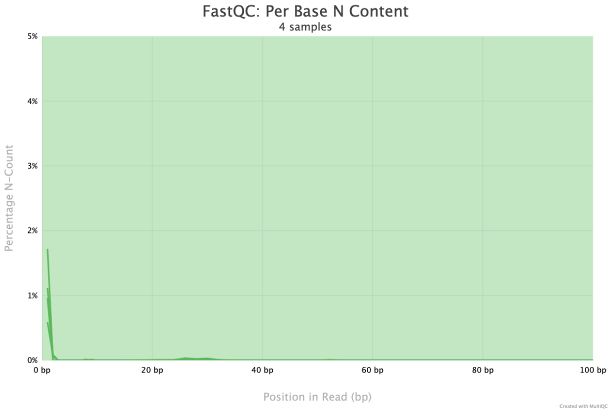


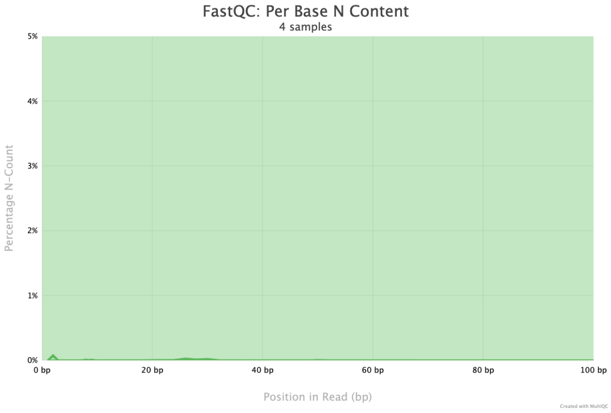
Raw Vs. Trimmed Adapter Content





Raw Vs. Trimmed Per Base N Content





Part 3

PS8 Script Outputs

29_4E_fox

```
Mapped                  8,883,008
Unmapped                260,800
```

Undetermined

```
Mapped                  15,584,503
Unmapped                8,735,639
```

htseq-count Outputs

29_4E_fox

Stranded=yes

```
    Mapped                  179,976
    No Feature              4,057,913
    Ambiguous               3,040
    Too Low Quality         8,168
    Not Aligned             126,013
    Alignment Not Unique    196,794
```

Stranded=reverse

```
    Mapped                  3,859,630
    No Feature              302,549
    Ambiguous               78,750
    Too Low Quality         8,168
    Not Aligned             126,013
    Alignment Not Unique    196,794
```

Undetermined

Stranded=yes

```
    Mapped                  292,237
    No Feature              7,078,461
    Ambiguous               5,990
    Too Low Quality         79,473
    Not Aligned             4,325,477
    Alignment Not Unique    378,433
```

Stranded=reverse

```
    Mapped                  6,616,501
    No Feature              629,222
    Ambiguous               130,965
    Too Low Quality         79,473
    Not Aligned             4,325,477
    Alignment Not Unique    378,433
```

Library Discussion

These reads are from “strand-specific” RNA-Seq Libraries, because we can observe a difference in mapping counts depending on how htseq-count’s stranded flag is set. When the flag is set to “stranded=yes”, the software checks that reads are in the same oreintation as the supplied transcripts. If this were the case for our datasets, we should observe higher feature mapping counts when the stranded flag is set to “yes”. Instead, we see drastically higher feature mapping counts when the flag is set to “reverse”. This is supported by the fact that Illumina’s TruSeq protocol produces libraries that are in reverse oreintation to feature transcripts.