FastQC QAA

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The objectives of this assignment are to: 1. Evaluate quality of RNA-Seq data using FastQC before and after removing adapters and trimming low quality reads. 2. Compare custom python script to the output of FastQC. 3. Connect concepts and tools developed in previous assignments. 4. Determine and justify stranded-ness of the data.

Data files assigned to process (R1 and R2): - 27_4C_mbnl_S19_L008
- 32_4G_both_S23_L008

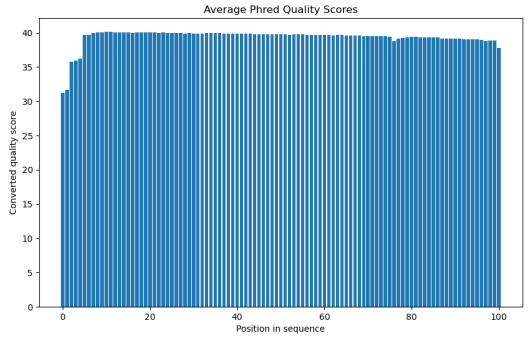
The plots I generated are basically the same in terms of what information they displey, however the FastQc plots include error bars, which might be variance or interquartile range.

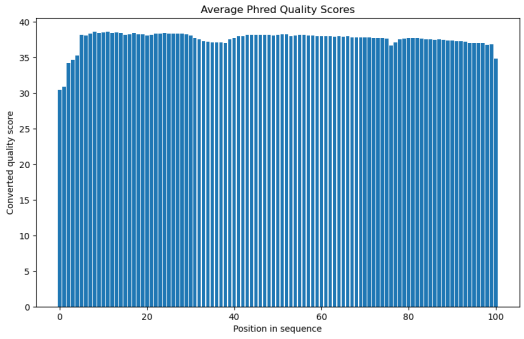
The read 1 files' quality is much better than the read 2s' quality score but that is due to the reads being later in the sequencing run.

Overall, this data seems to be of good quality, none of the error bars of fastqc output seem to have dipped into the red regions so that must at least be sufficient quality to do something with.

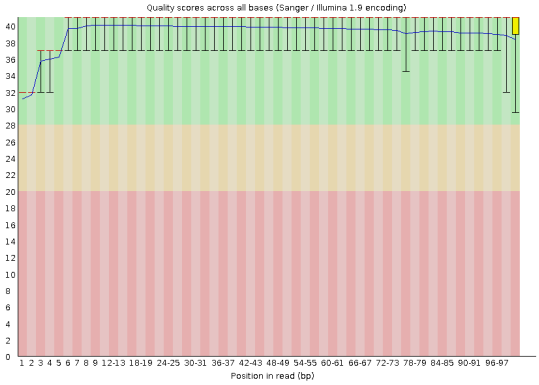
FastQC outputs from raw files

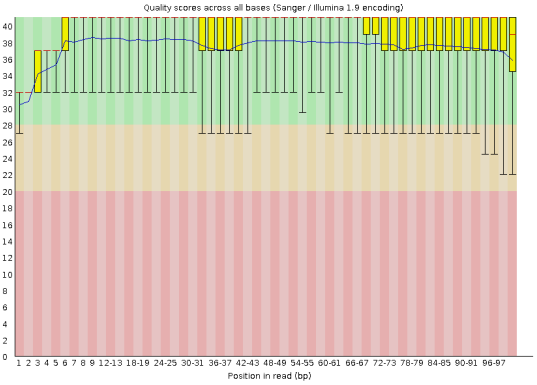
A



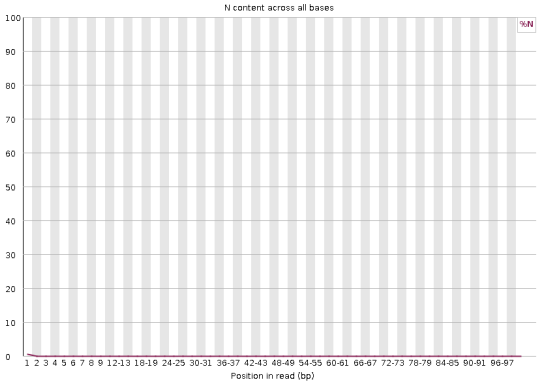


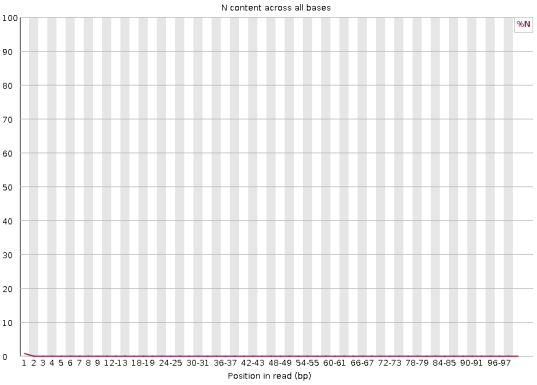
B



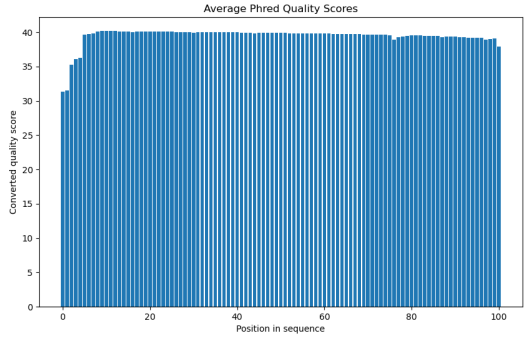


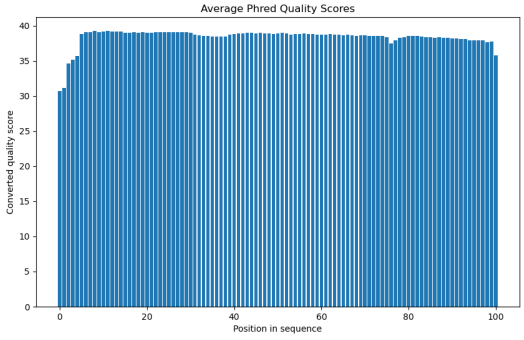
C



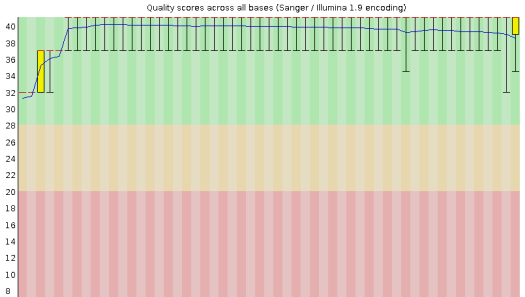


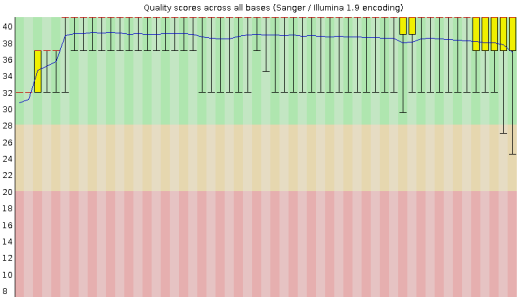
A





B





Figures 1 and 2 contain FastQC quality data for both samples 27_4C_mbnl_S19_L008 and 32_4G_both_S23_L008 respectively. Row A contains plots generated by custom plotting script `mean_qual.py`. Row B is the same data, produced by FastQC. See table below for further differences between these plots. Row C contains the per-base N content for each read file. The columns in these figures correspond to R1 and R2 reads.

Table of the times and memory for each of these commands as timed with `/usr/bin/time -v`

File	FastQC Time	FastQC Memory (kbytes)	mean_qual.py Time	mean_qual.py Memory (kbytes)
27_4C_mbnl_S19_L008_R1	0:31.36	370344	1:36.44	65688
27_4C_mbnl_S19_L008_R2	0:30.18	367432	1:29.58	65612
32_4G_both_S23_L008_R1	0:46.33	345816	2:26.40	63568
32_4G_both_S23_L008_R2	0:46.21	349428	2:26.72	65540

FastQC is much faster (it is calculating so much more) than my python script. The graphs are similar however, with mine only missing error bars (IQR).

Trimming

After these first plots, data are adapter trimmed using `cutadapt` and then quality trimmed with `trimmomatic`. Using another custom pyplot script, plot the distributions of read lengths also.

Cutadapt information: - Sample 27_4C_mbnl_S19_L008 - Total read pairs processed: 7,226,430 - Read 1 with adapter: 751,117 (10.4%) - Read 2 with adapter: 803,568 (11.1%) - Pairs written (passing filters): 7,226,430 (100.0%) - Sample 32_4G_both_S23_L008 - Total read pairs processed: 11,820,174 - Read 1 with adapter: 631,720 (5.3%) - Read 2 with adapter: 725,571 (6.1%) - Pairs written (passing filters): 11,820,174 (100.0%)

FastQC outputs from trimmed files

Figures 3 and 4 contain FastQC output of mean quality score by base position for samples 27_4C_mbnl_S19_L008 and 32_4G_both_S23_L008 respectively. Row A shows the plots for R1 and R1 (left and right respectively) before trimming (raw reads). Row B contains plots of trimmed data.

Trimmed files length distributions

In Figure 5, graph A corresponds to sample 27_4C_mbnl_S19_L008.Rlendist.png, graph B corresponds to sample 32_4G_both_S23_L008.Rlendist.png. These reads have been adapter cut and quality trimmed.

Create an alignment database for *M. musculus* using STAR RNA-Seq aligner. Align both samples to this database. Using `htseq-count`, reads which mapped to features were counted for both `--stranded=yes` and `--stranded=reverse`

Results of STAR alignment:

Sample	Reads Mapped	Reads Not Mapped
27_4C_mbnl_S19_L008	13320032	433878
32_4G_both_S23_L008	22404331	533601

The data are from strand specific RNA-Seq libraries. The vast majority of reads (82.701%, 86.4336%) were mapped using `--stranded=reverse`, while only 3.91303%, 3.82643% mapped using `--stranded=yes` for

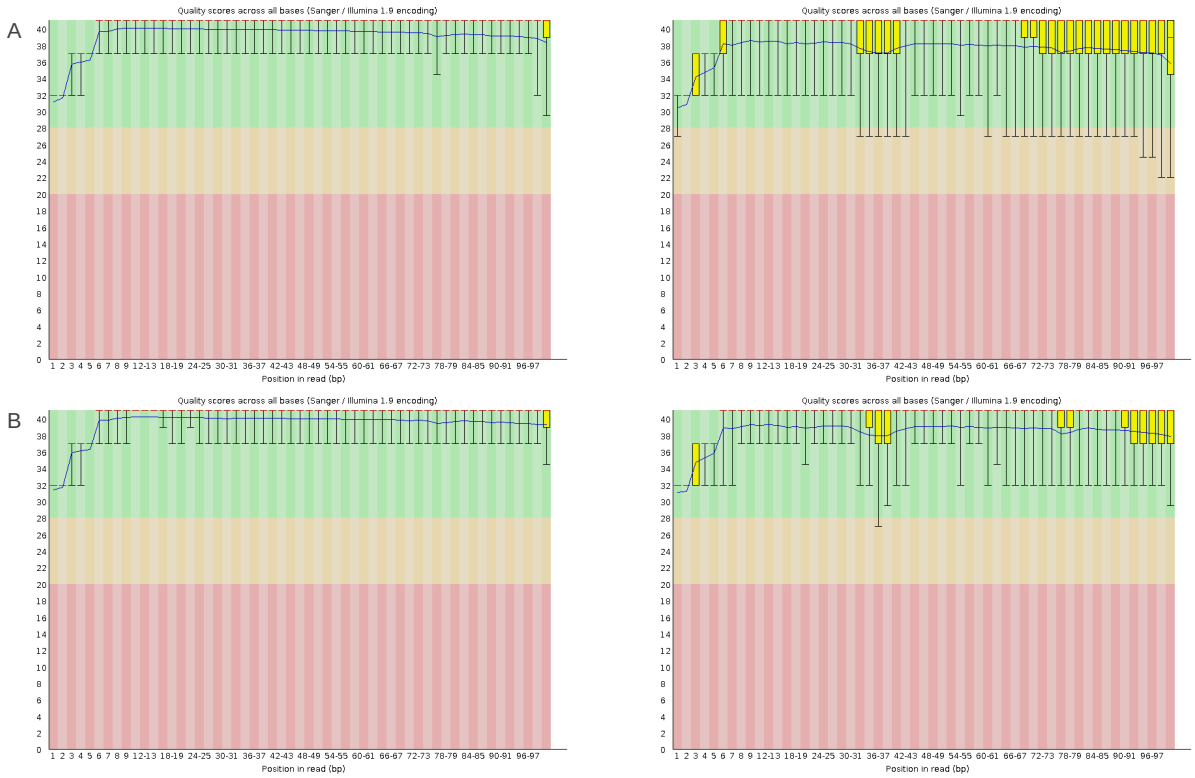


Figure 1: Fig. 3: Per Base Quality Score From FastQC

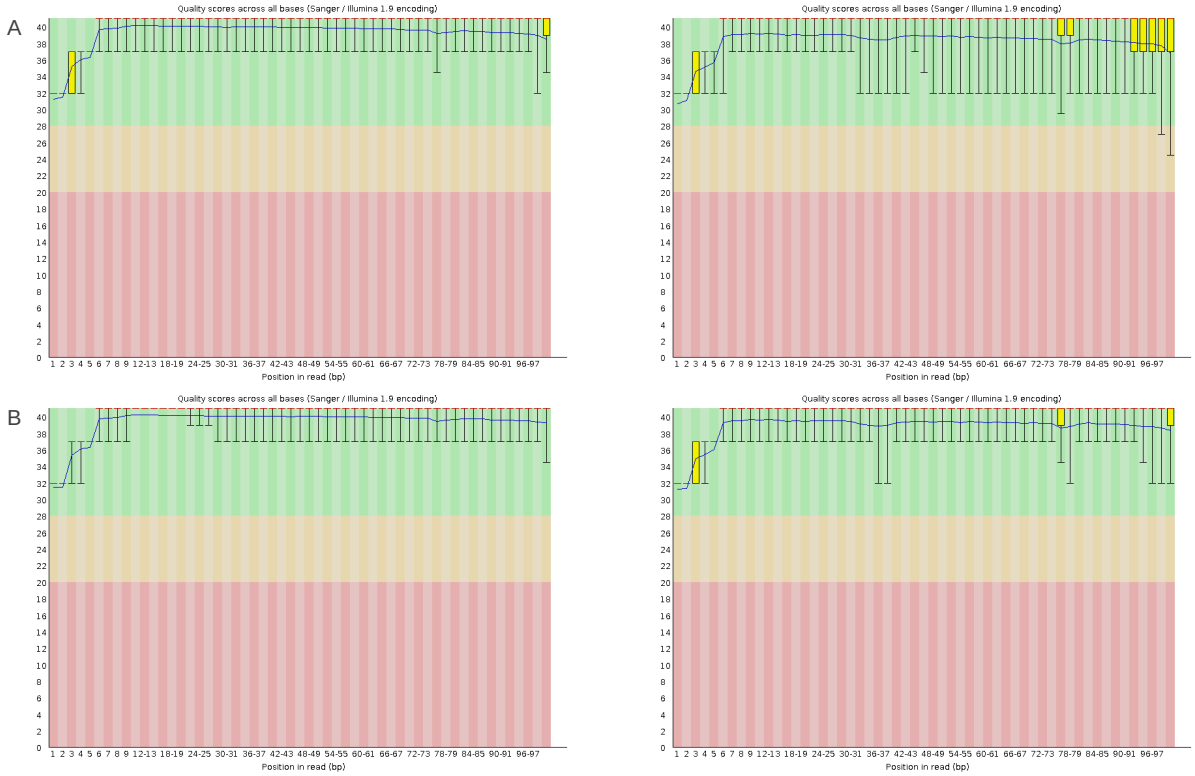


Figure 2: Fig. 4: Per Base Quality Score From FastQC

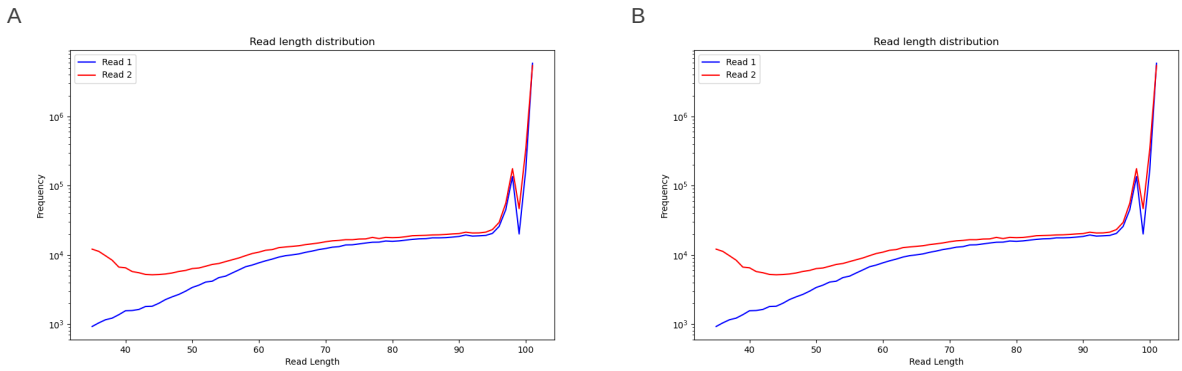


Figure 3: Fig. 5: Read Length Distributions

samples 27_4C_mbnl_S19_L008 and 32_4G_both_S23_L008 respectively. I used commands from ICA4 in Bi621.