GULBENKIAN SCIENCE

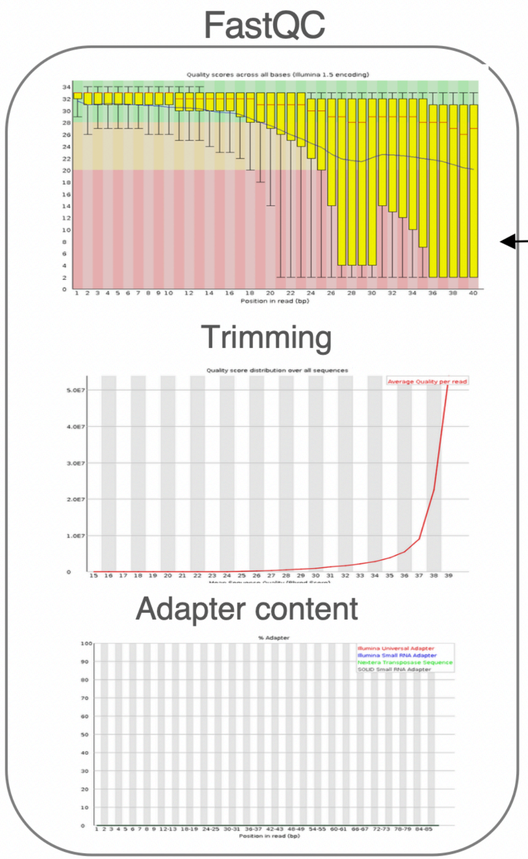
PO21: Precision Oncology Course

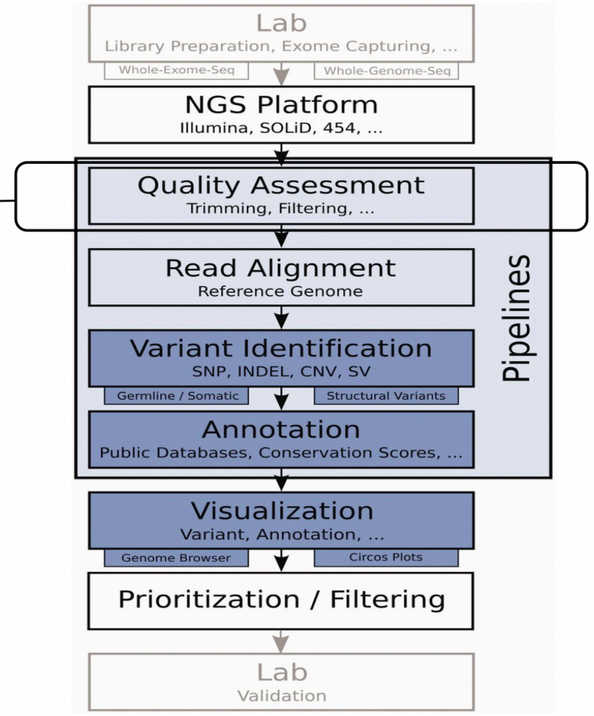
Quality Control

BU CNIO BIOINFORMATICS UNIT

cnio stop cancer

Remember





Stephan Pabinger et al. Brief Bioinform 2013;bib.bbs086

Quality Control (QC) of **raw sequencing data** is the **first step** in our bioinformatics workflow. It is done **to make sure** that **data is OK**.

Software for QC

FastQC and MultiQC

Software: FastQC

The QC is performed by **FastQC**, which takes a FASTQ as input and returns an HTML **web-like report with plots** as output.

Software: FastQC

```
@SRR6671104.1 SN7001303:484:hwkwybcxx:1:1103:1482:2094 length=101
NCCAAGCGTTCATAGCGACGTCGCTTTTTGATCCTTCGATGTCGGCTCTTCCTATCATTGTGAAGCAG
AATTCACCAAGCGTTGGATTGTTCACCCACTAA
+SRR6671104.1 SN7001303:484:hwkwybcxx:1:1103:1482:2094 length=101
#<DDDIIIHIIIIGHHIHIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIFHIIIIIIIIGHIHIIIHI
IIIIIIIIIIIIIIIIGIIIIIIIIIHIIIIIE
@SRR6671104.2 SN7001303:484:hwkwybcxx:1:1103:1407:2185 length=101
CACCAAAACCGTGAAGAAGGCGGCCCGGGTCATCATAGAAAAGTACTACACGCGCCTGGGCAACGACT
TCCACACGAACAAGCGCGTGTGCGAGGAGATCG
+SRR6671104.2 SN7001303:484:hwkwybcxx:1:1103:1407:2185 length=101
DDDDDIIIIIIIIIIIIIIIIIIIIIIIIIIIHIIIIIIIIIIIIIIIIIIIIIIIIGIIIIIIIIII
IIIHIIIIIIIIIIIIIIIIIIIIIIICHHIGIG
@SRR6671104.3 SN7001303:484:hwkwybcxx:1:1103:1363:2198 length=101
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CCGTGGAGGCAGTGGCTGTGGGCATAGTGGCCA
+SRR6671104.3 SN7001303:484:hwkwybcxx:1:1103:1363:2198 length=101
DDDDDIIHHIIHIGHIIIEHCHFHIIIIGIIGEHG@EIIIIHDHHHHHIGGCDCGHHHHIIHIGFEHH
HEHIHHGHHIFHHHEHGGHIIIHIHIIIIIHE@
@SRR6671104.4 SN7001303:484:hwkwybcxx:1:1103:1399:2209 length=101
GTCAGTGTAGCGCGCGTTCAGCCACCCGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGATGTCC
GGGGCTGCACGCGCGCTACACTGACTGGCTCAG
+SRR6671104.4 SN7001303:484:hwkwybcxx:1:1103:1399:2209 length=101
DDDDDHHHIHHIIIIHHDHIHEHIIHHHDCCGHIIGHIIHIIIIHHHHIIHHEHIEHHEHCHHIHIIH
HHHIIIGIH?CCEHCHHIIIHIIII?1F1FGH
@SRR6671104.5 SN7001303:484:hwkwybcxx:1:1103:1344:2225 length=101
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GCTATATGAAATATGGGAGACGACAGACCGTTT
```
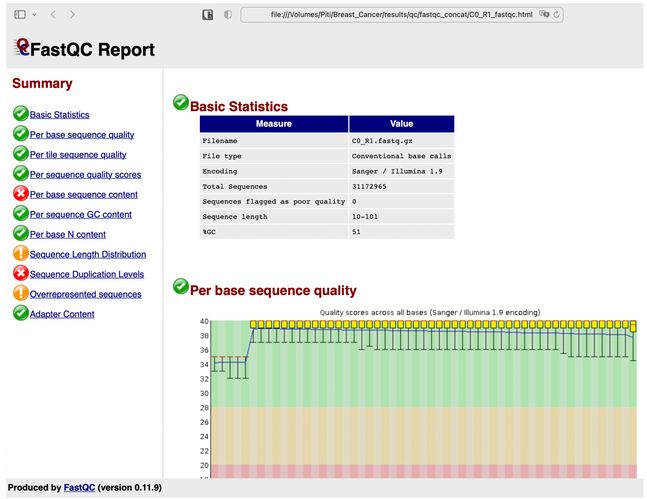
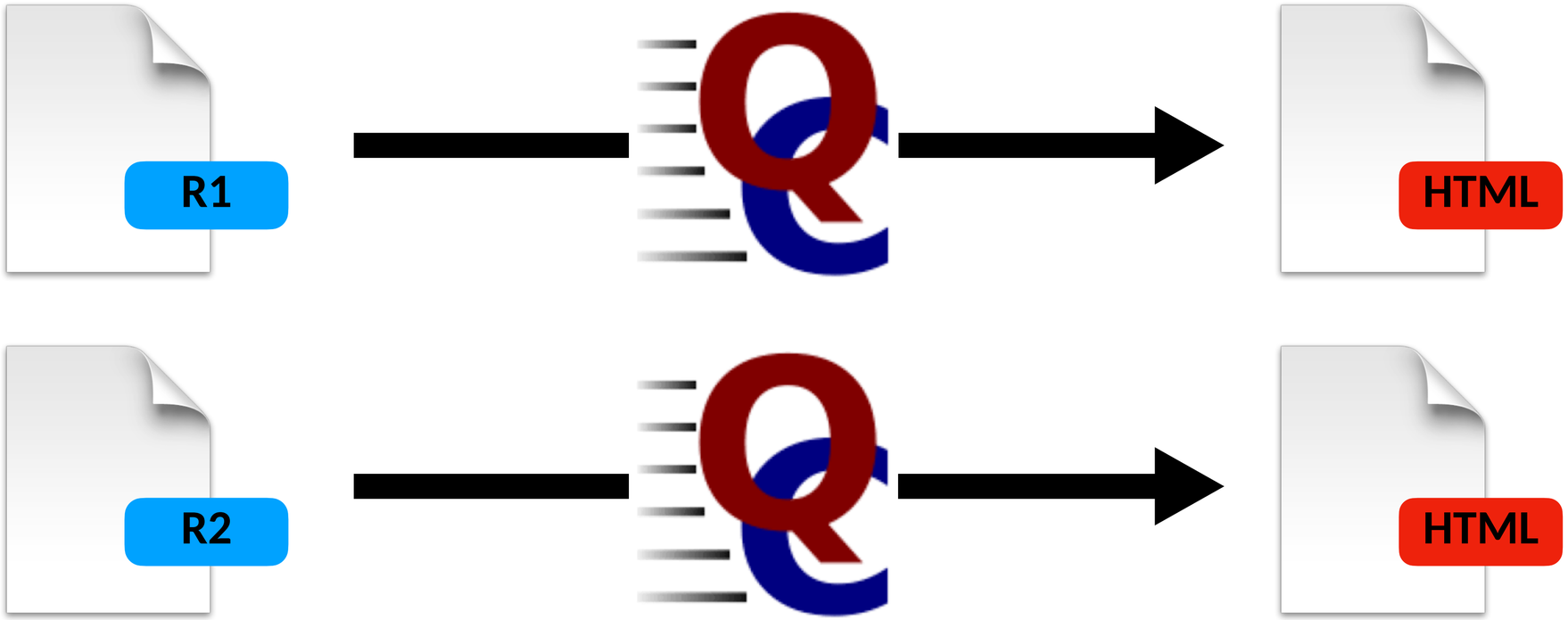



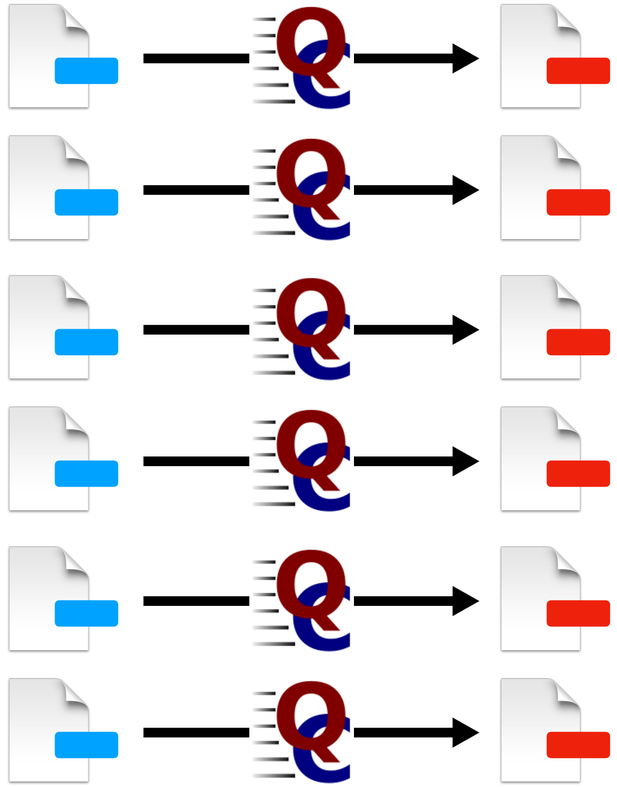
The QC is performed by **FastQC**, which takes a FASTQ as input and returns an HTML **web-like report with plots** as output.

Software: FastQC



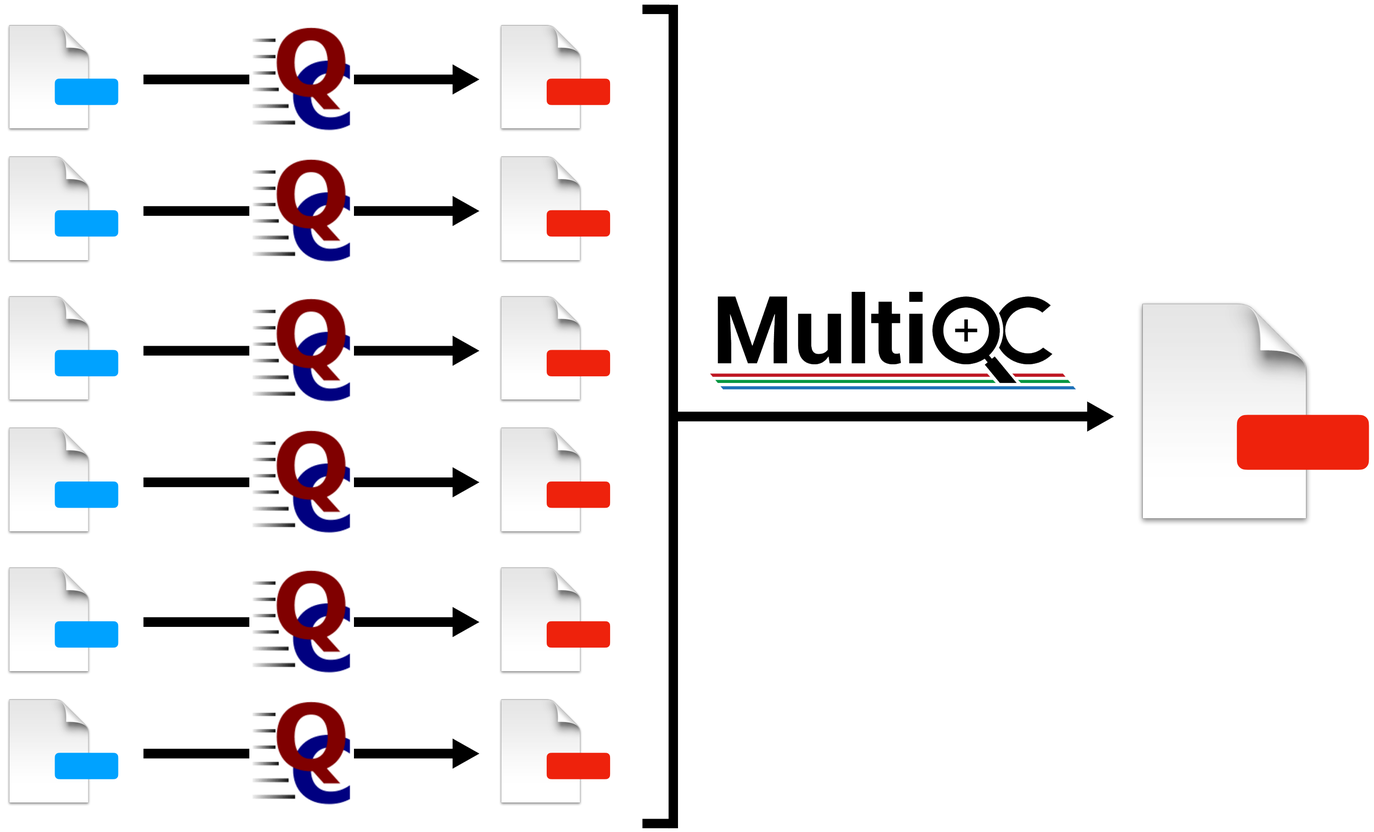
If you have **paired-end sequencing** data (two FASTQs per sample: R1.fastq and R2.fastq) **you will get two reports per sample.**

Software: FastQC



In a normal analysis you will end up with **a lot of reports**.

Software: MultiQC

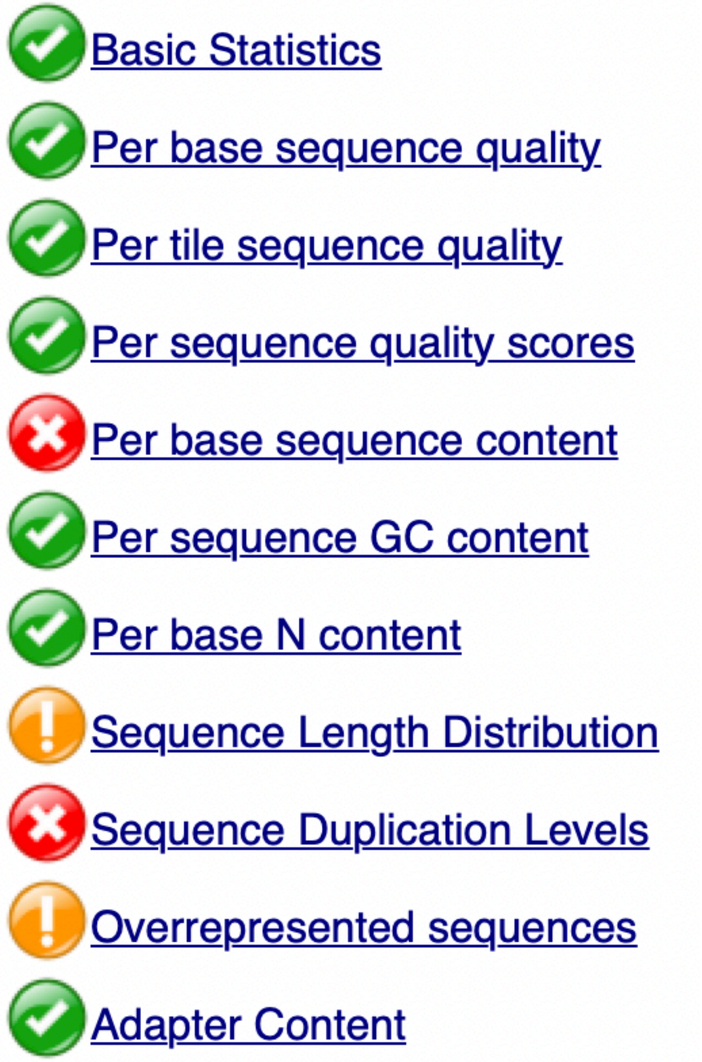


MultiQC is a program that **aggregates all FastQC reports** into a single **interactive** one.

Report sections in FastQC and MultiQC

Examples of Good and Bad results

FastQC report sections



- The results seem normal
- The results seem slightly abnormal
- The results seem unusual

Report sections

- Basic Statistics
- Per base sequence quality
- Per tile sequence quality
- Per sequence quality scores
- Per base sequence content
- Per sequence GC content
- Per base N content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content

MultiQC

- Sequence Counts
- Sequence Quality Histograms
- Per Sequence Quality Scores
- Per Base Sequence Content
- Per Sequence GC Content
- Per Base N Content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content
- Status Checks

Report sections

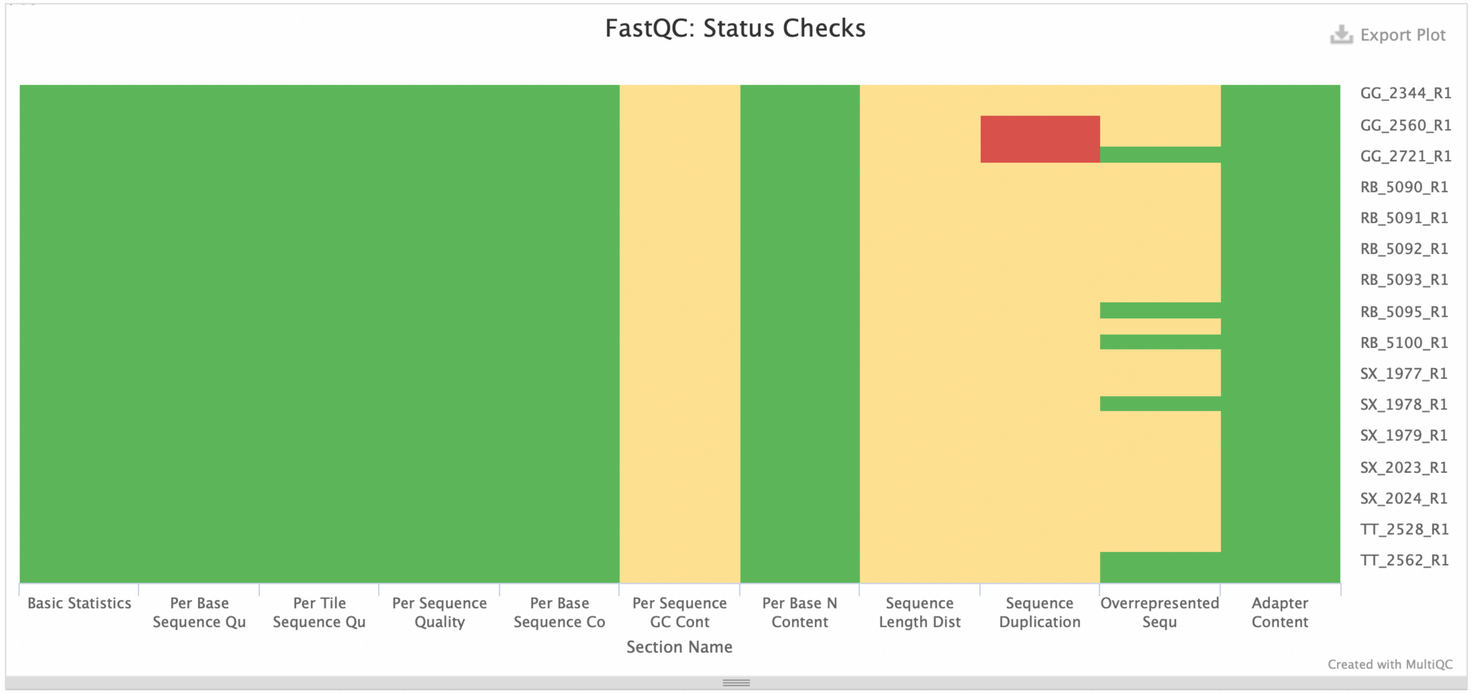
- Basic Statistics
- Per base sequence quality
- Per tile sequence quality
- Per sequence quality scores
- Per base sequence content
- Per sequence GC content
- Per base N content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content

Status Checks

Help

Status for each FastQC section showing whether results seem entirely normal (green), slightly abnormal (orange) or very unusual (red).

Sort by highlight



FastQC Basic Statistics

Basic Statistics

Measure	Value
Filename	20210722_2344_AM9009_S75_R1_001.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	59796259
Sequences flagged as poor quality	0
Sequence length	150
%GC	45

Basic Statistics

Basic Statistics

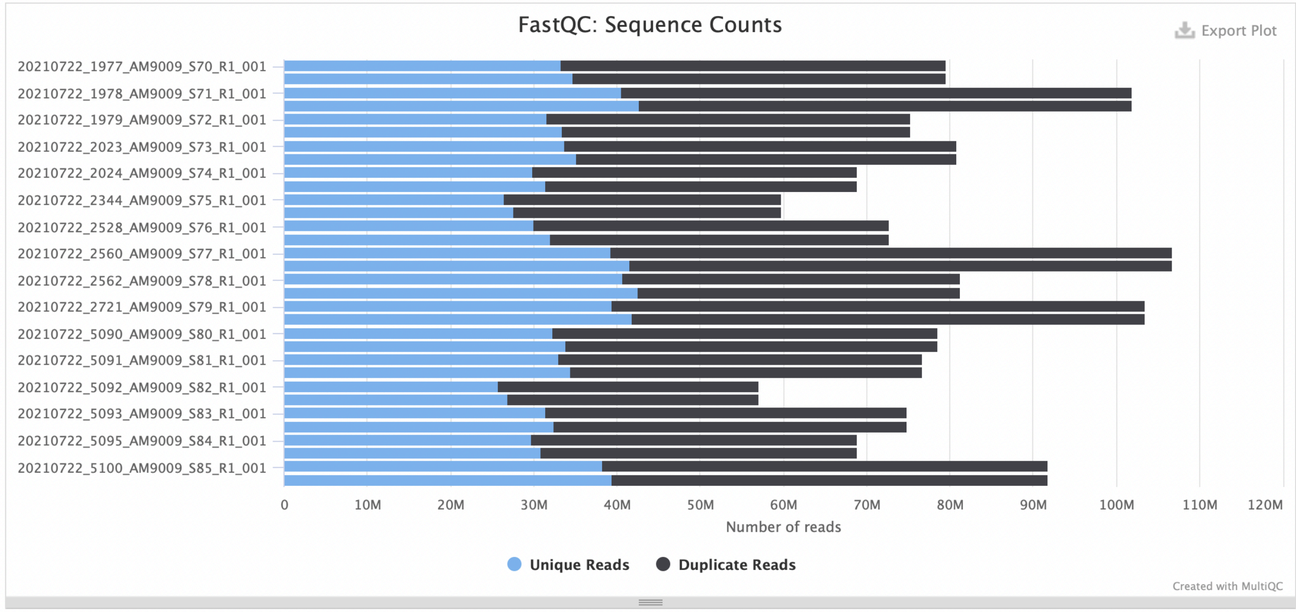
Measure	Value
Filename	20210722_2344_AM9009_S75_R1_001.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	59796259
Sequences flagged as poor quality	0
Sequence length	150
%GC	45

Sequence Counts

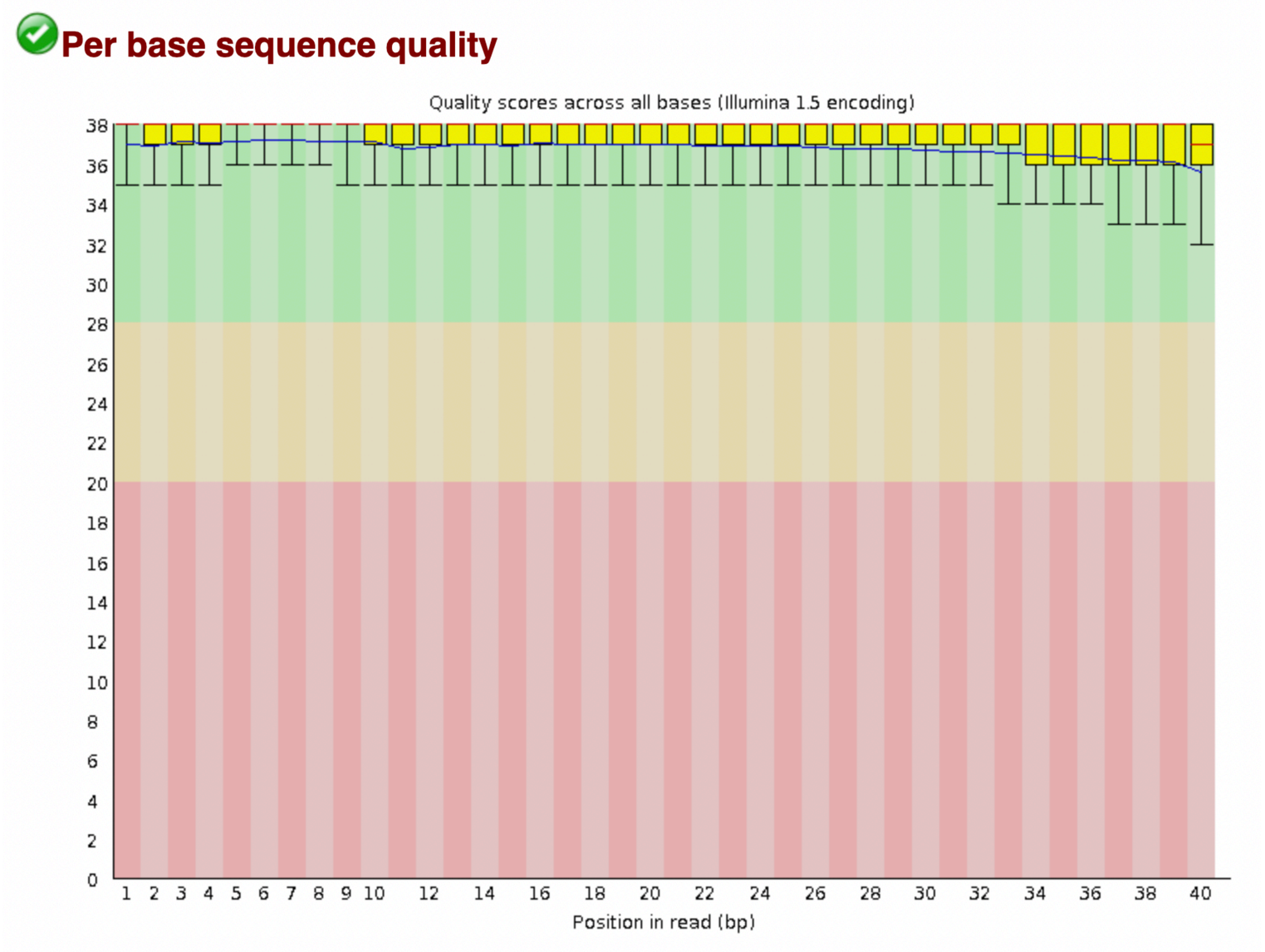
Help

Sequence counts for each sample. Duplicate read counts are an estimate only.

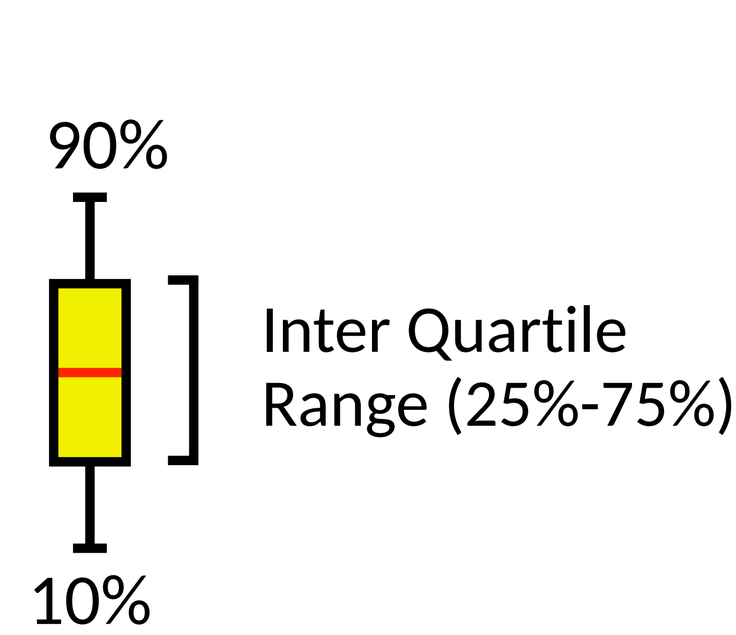
Number of reads | Percentages



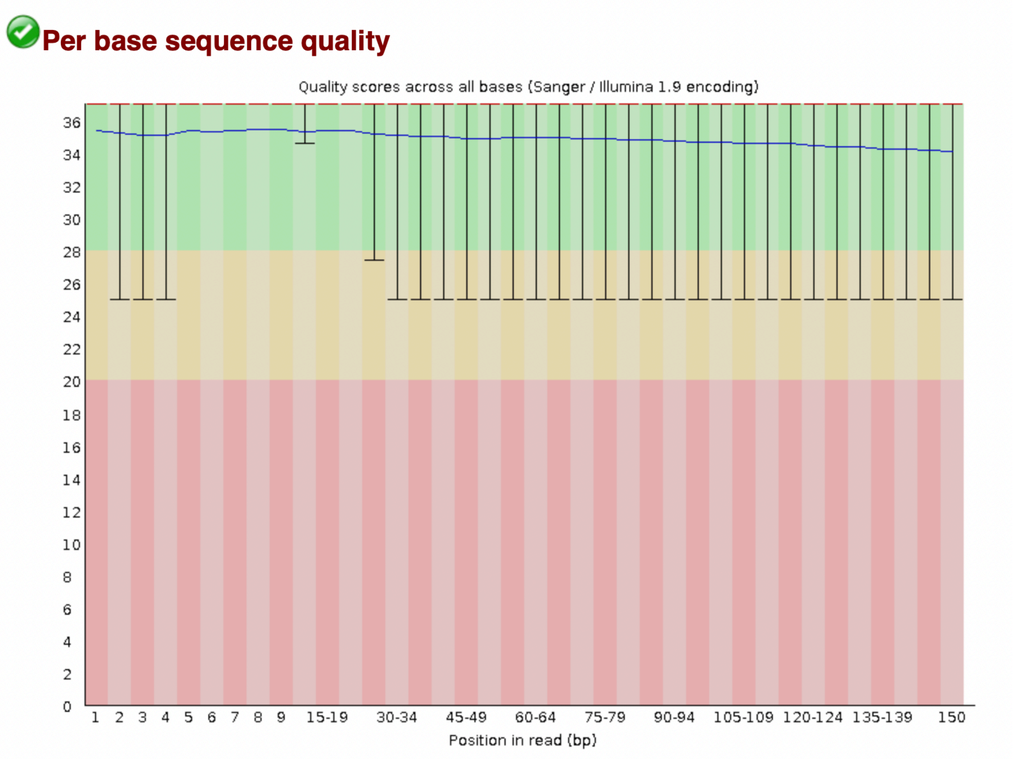
FastQC Per Base Quality

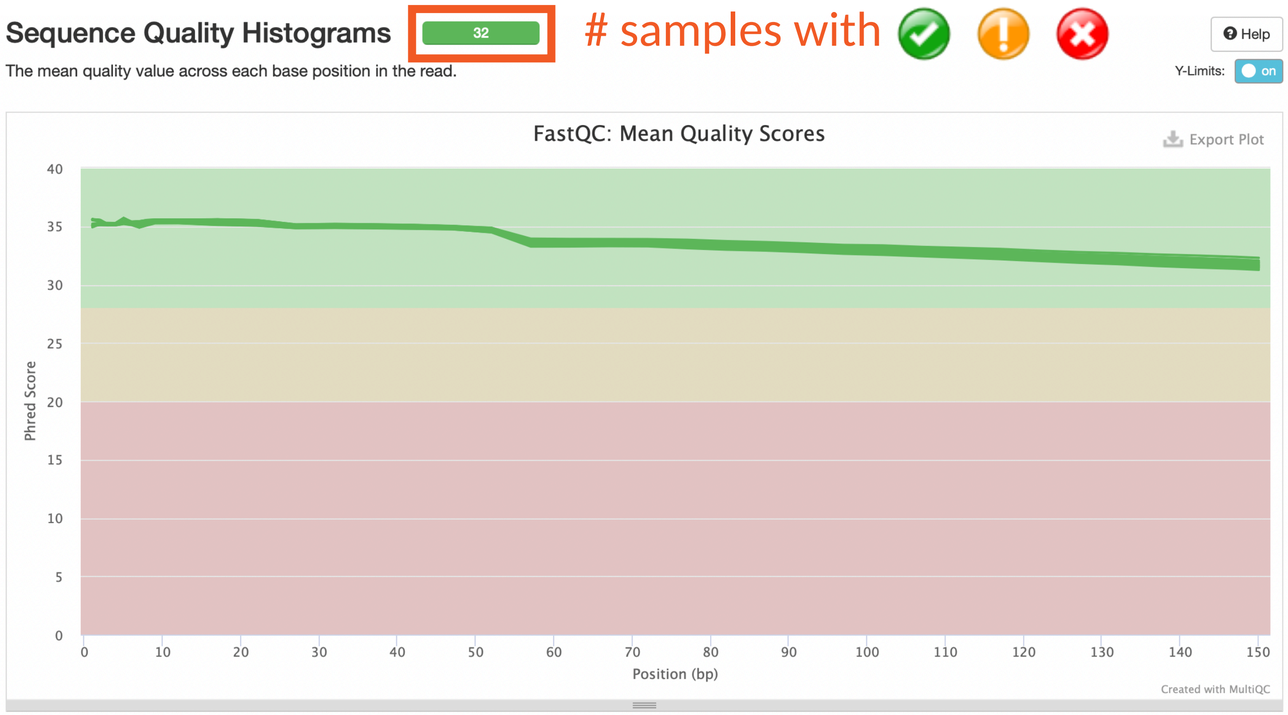


FastQC Example Reports: Good Illumina Data



Per Base Quality

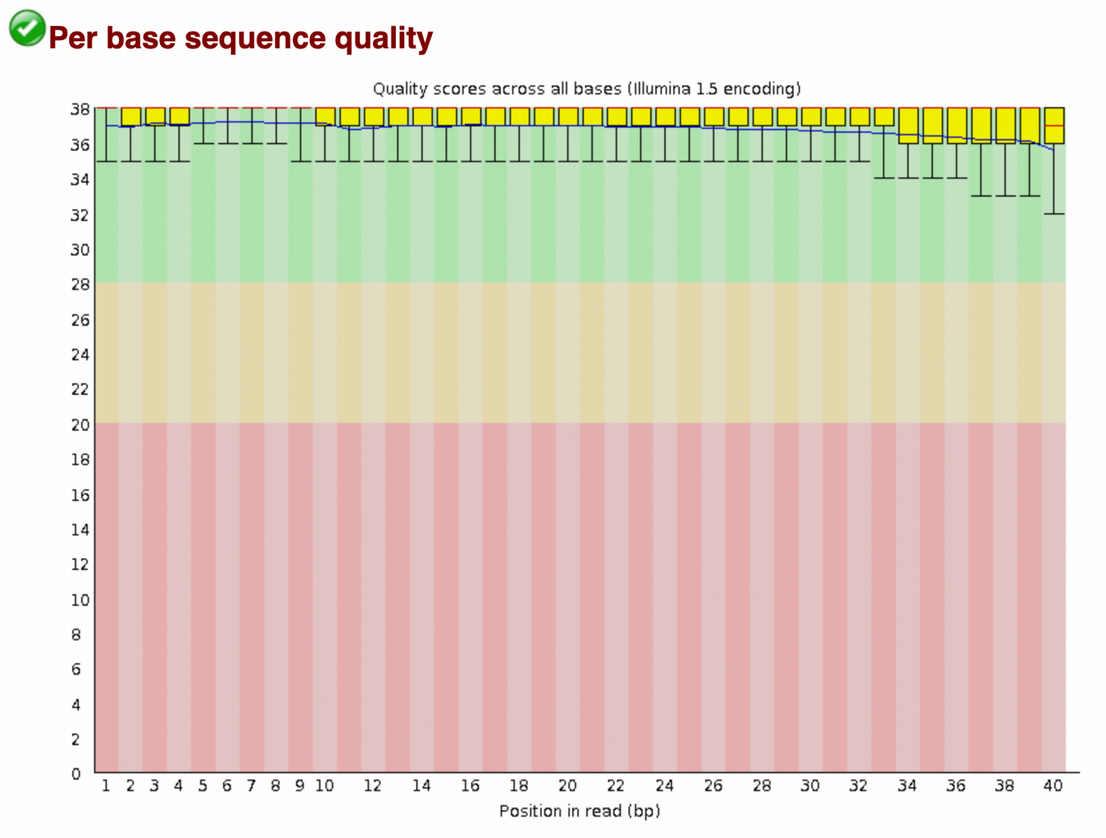




Usually, long reads lose quality at the end.

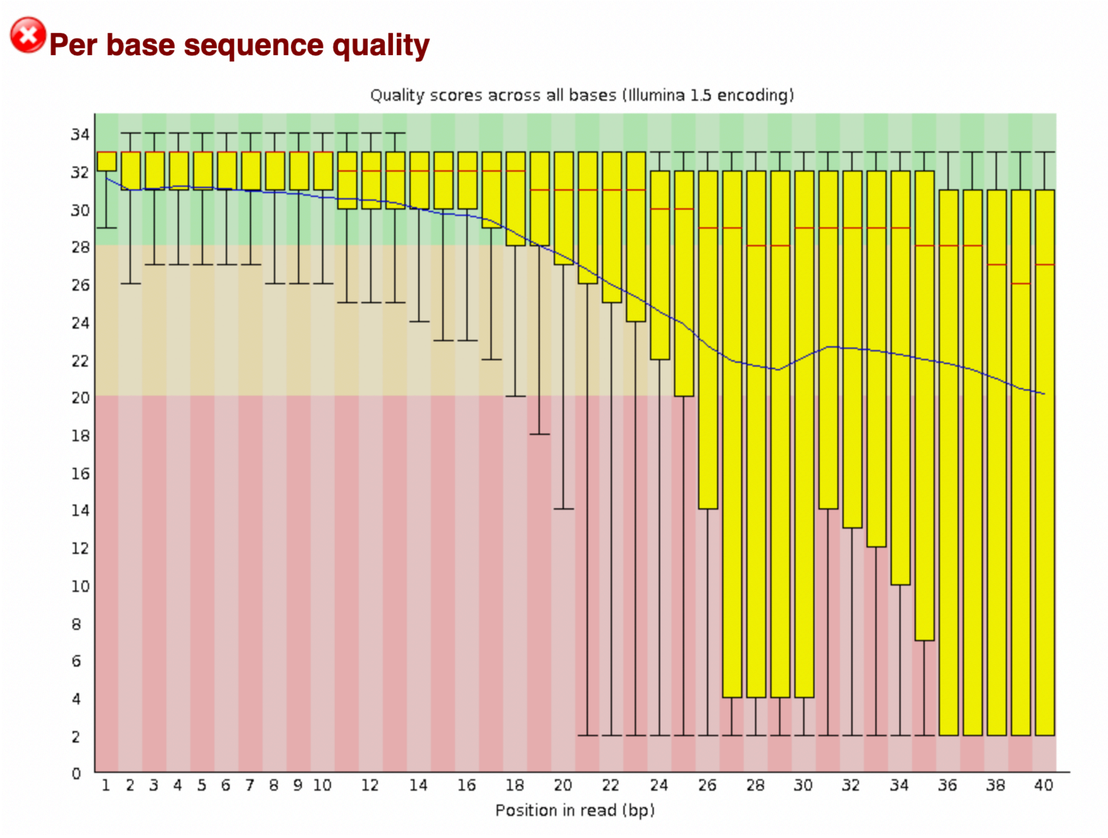
FastQC Per Base Quality

Good result



FastQC Example Reports: Good Illumina Data

Bad result

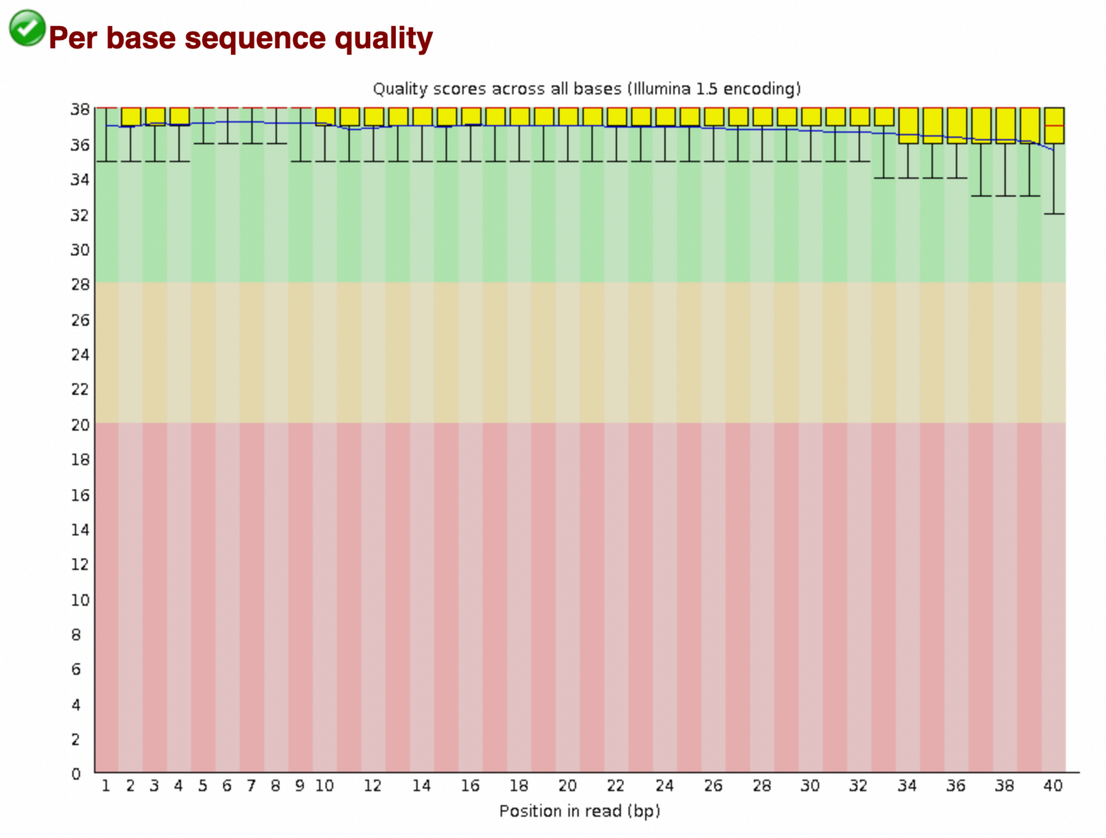


FastQC Example Reports: Bad Illumina Data

Solution: Trim were the reads are truncated based on their average quality.

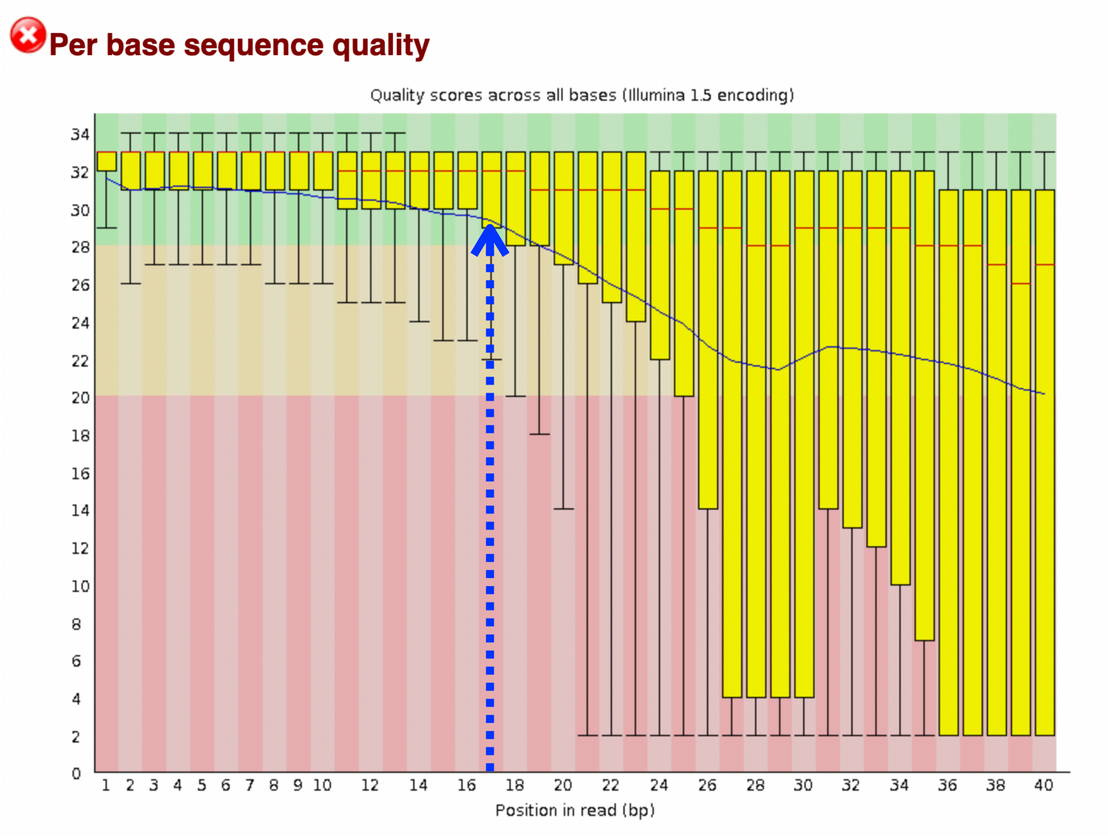
FastQC Per Base Quality

Good result



FastQC Example Reports: Good Illumina Data

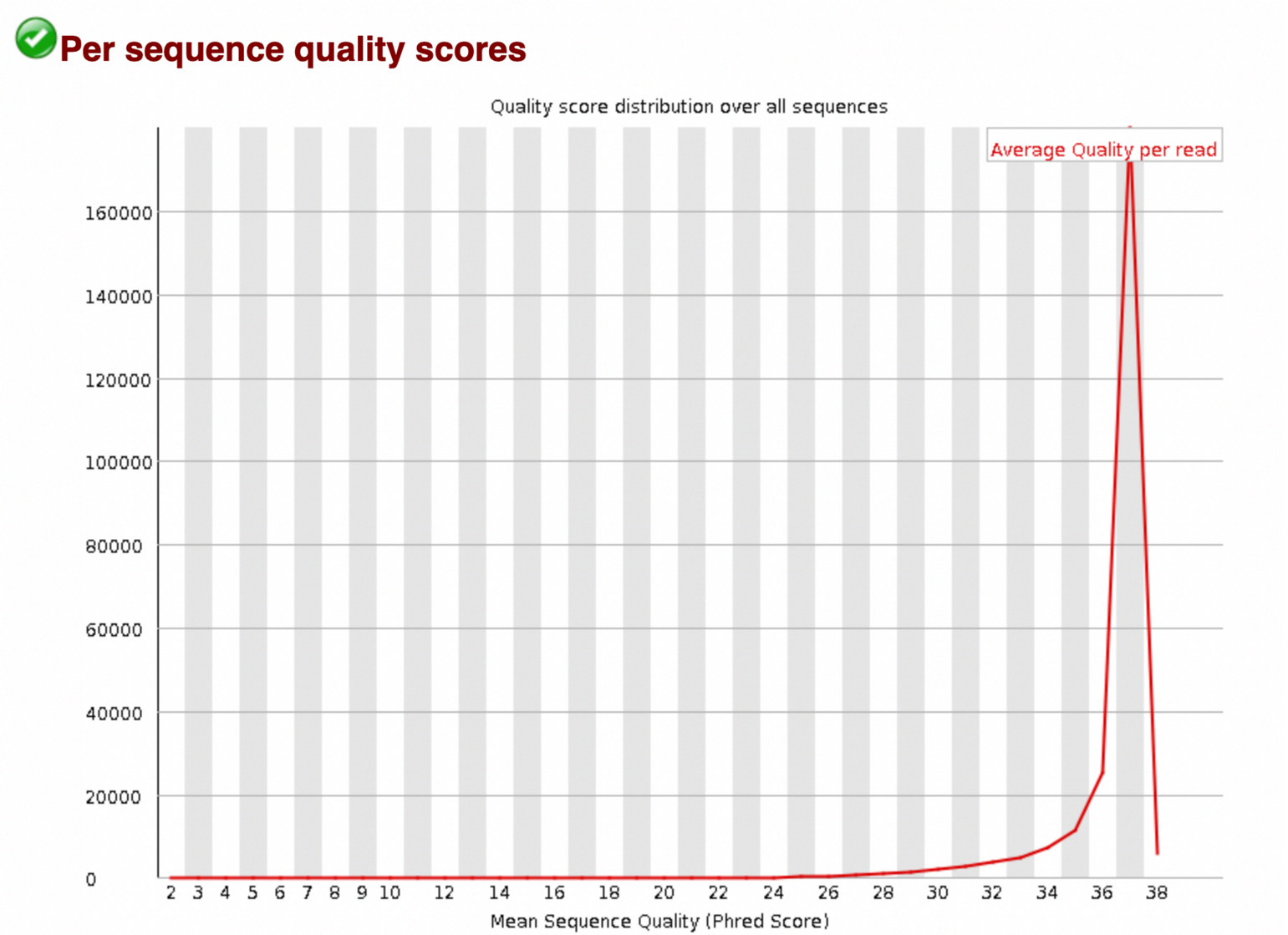
Bad result



FastQC Example Reports: Bad Illumina Data

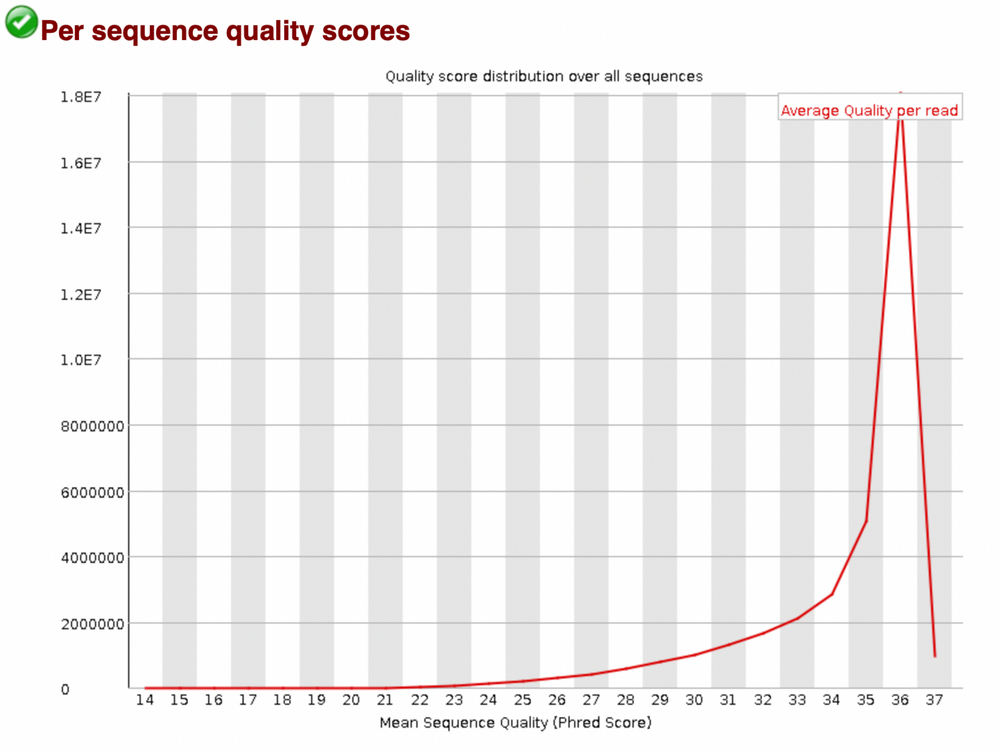
Solution: Trim were the reads are truncated based on their average quality.

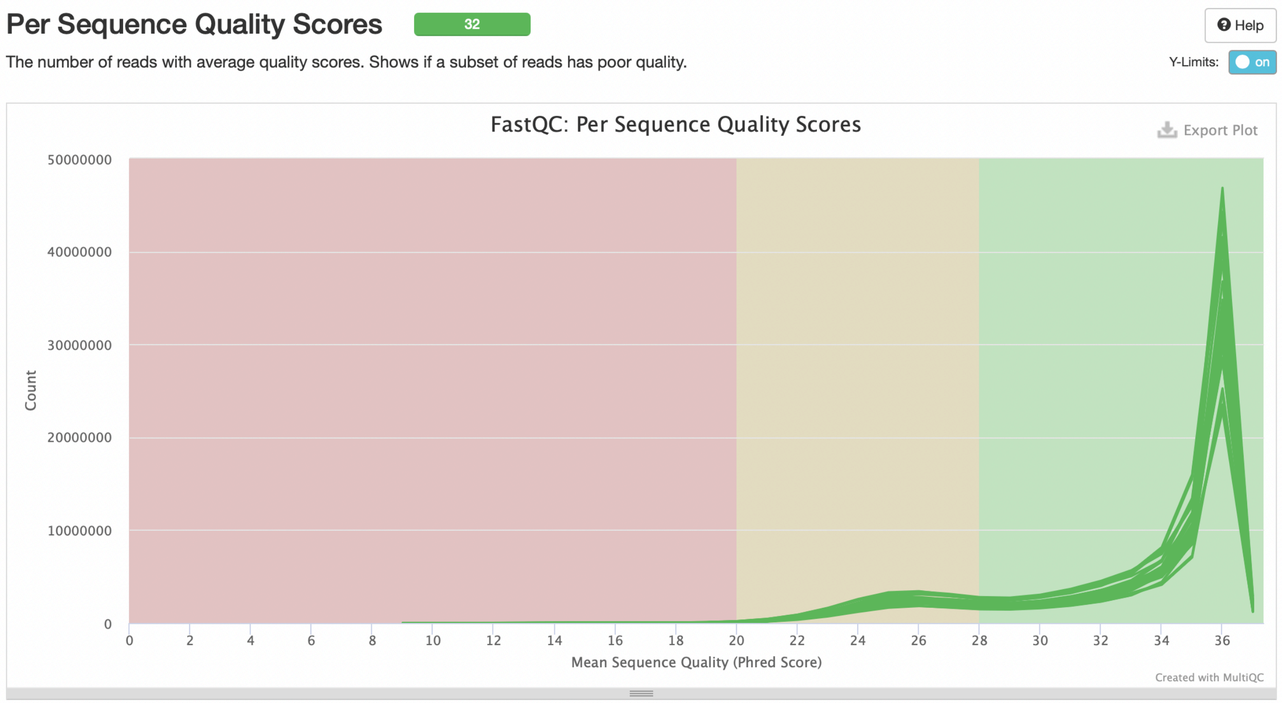
FastQC Per Sequence Quality Scores



FastQC Example Reports: Good Illumina Data

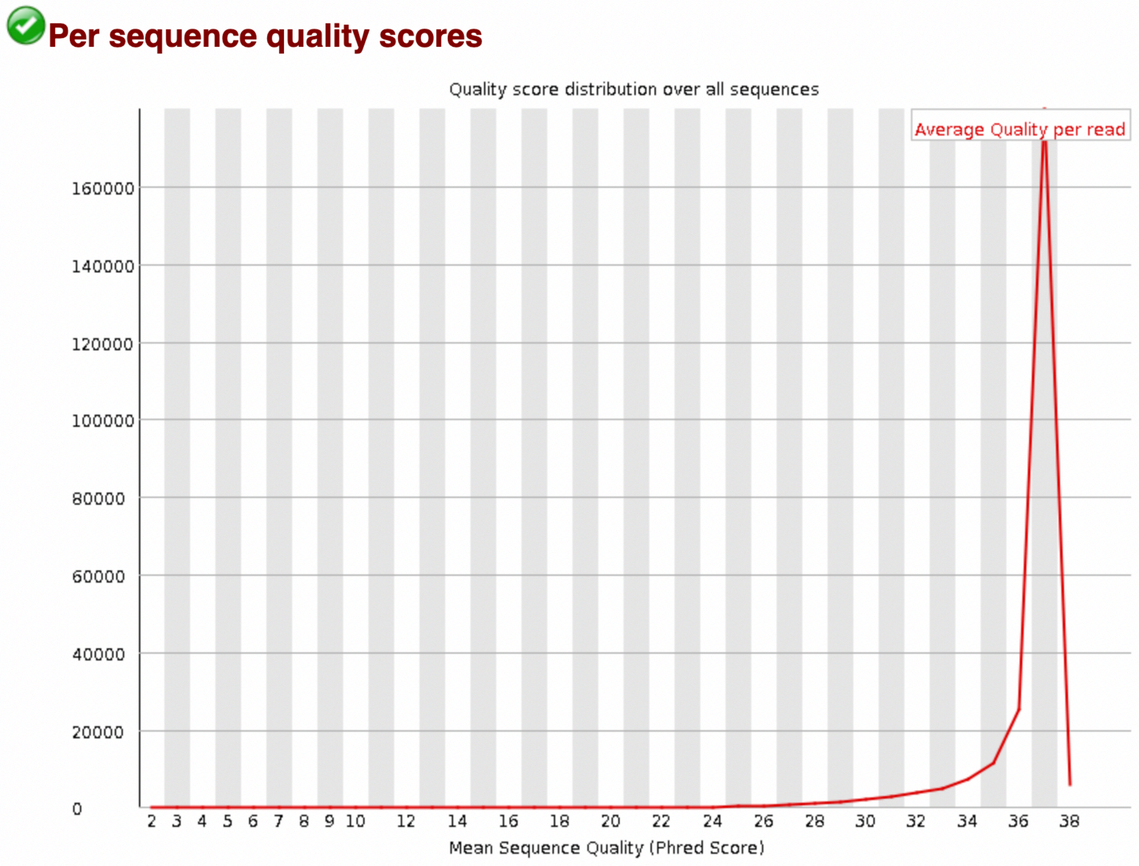
Per Sequence Quality Scores





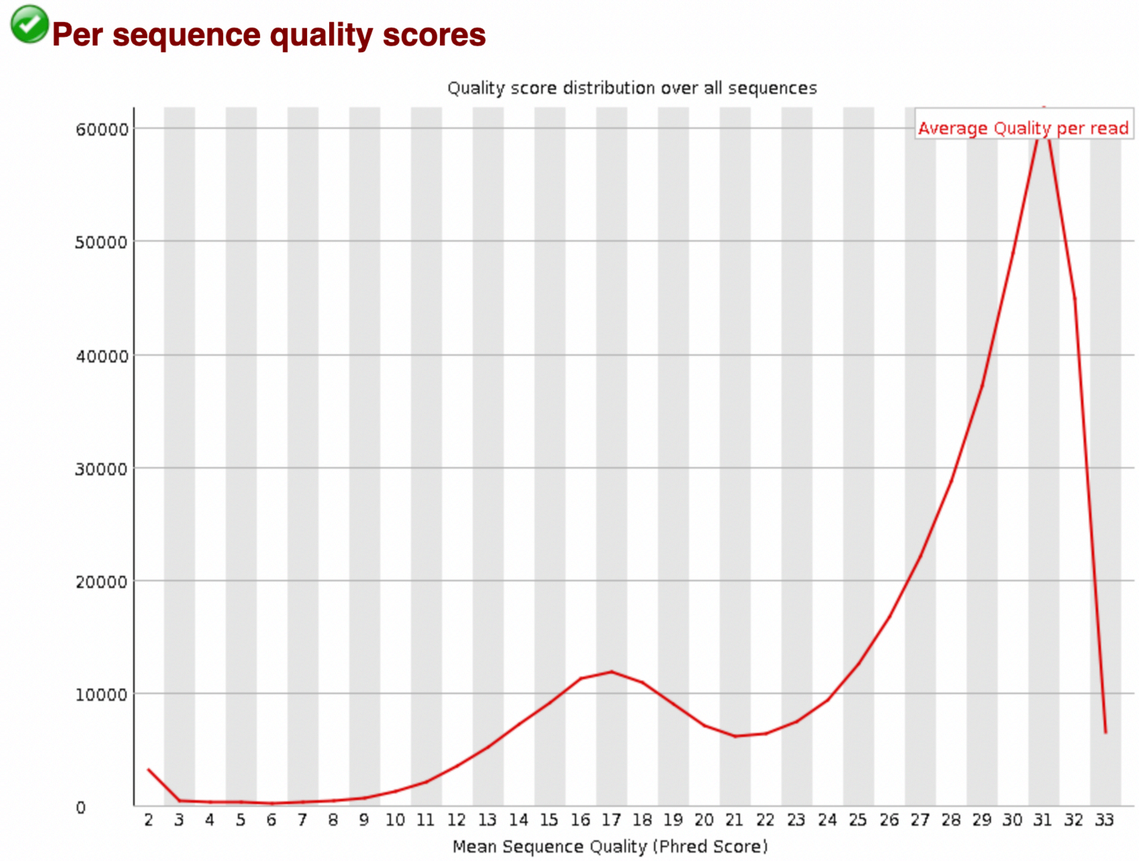
FastQC Per Sequence Quality Scores

Good result



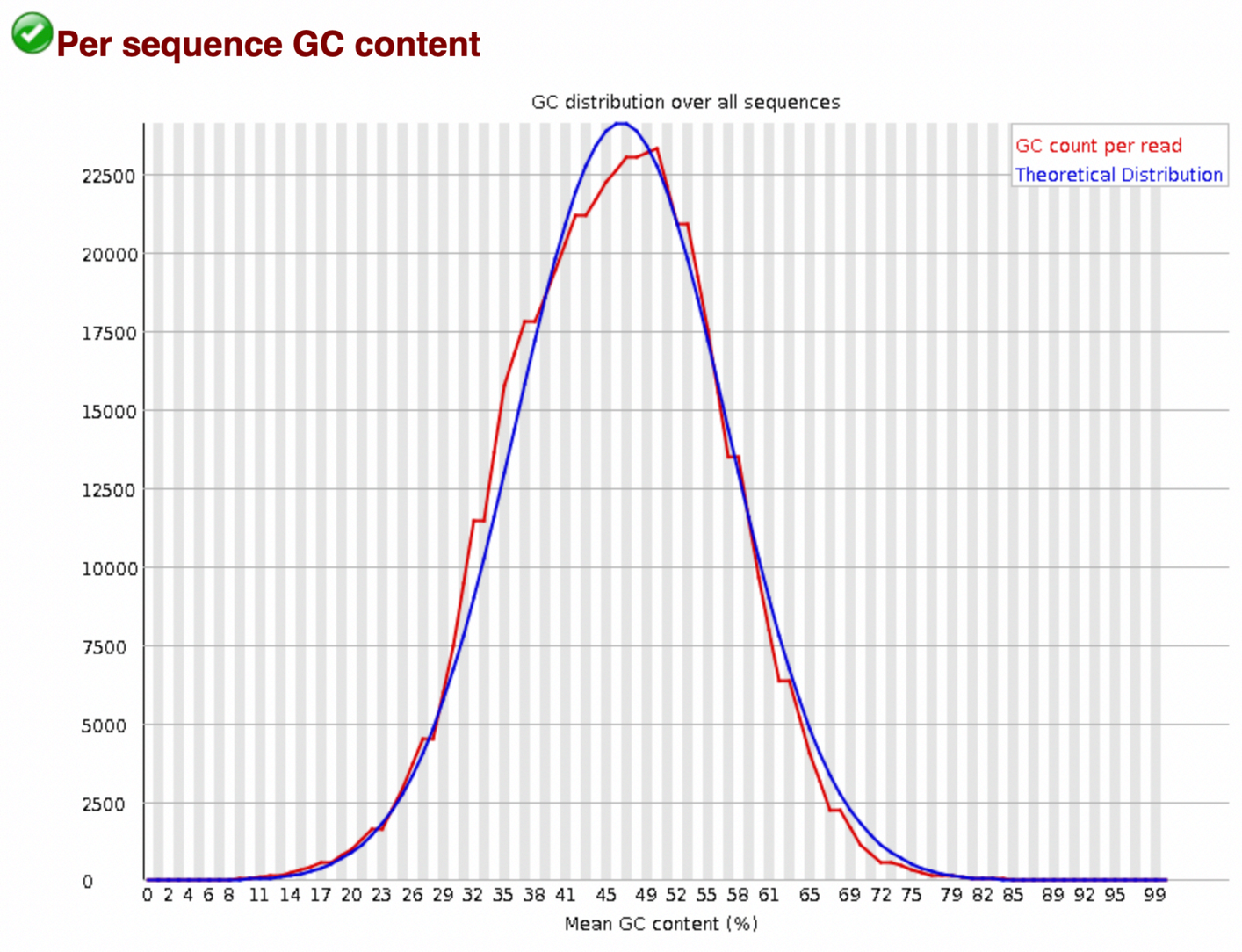
FastQC Example Reports: Good Illumina Data

Bad result



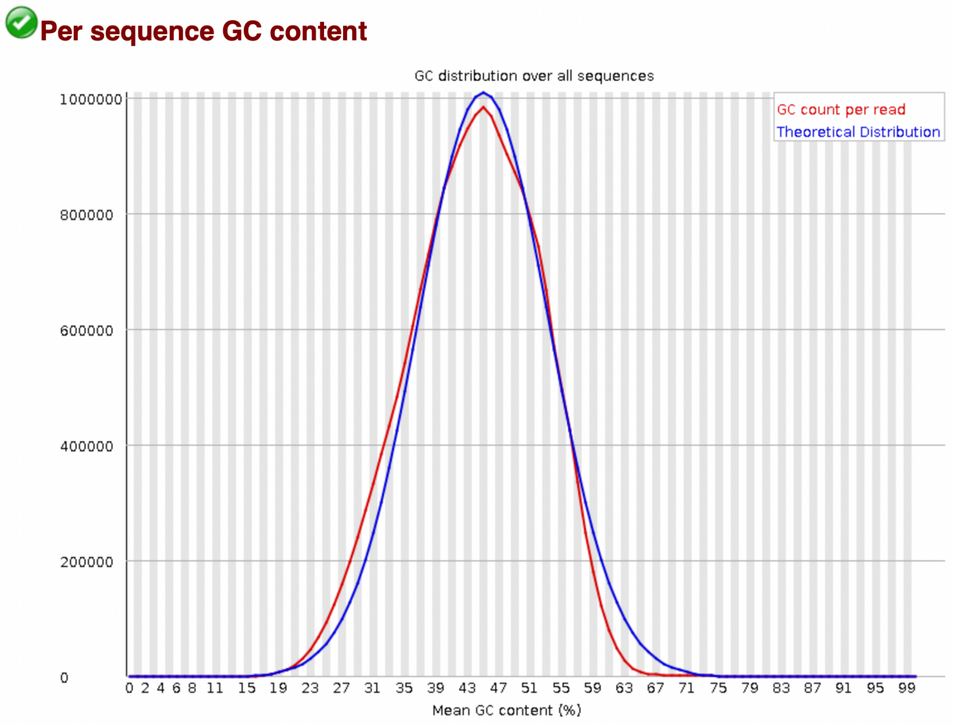
FastQC Example Reports: Bad Illumina Data

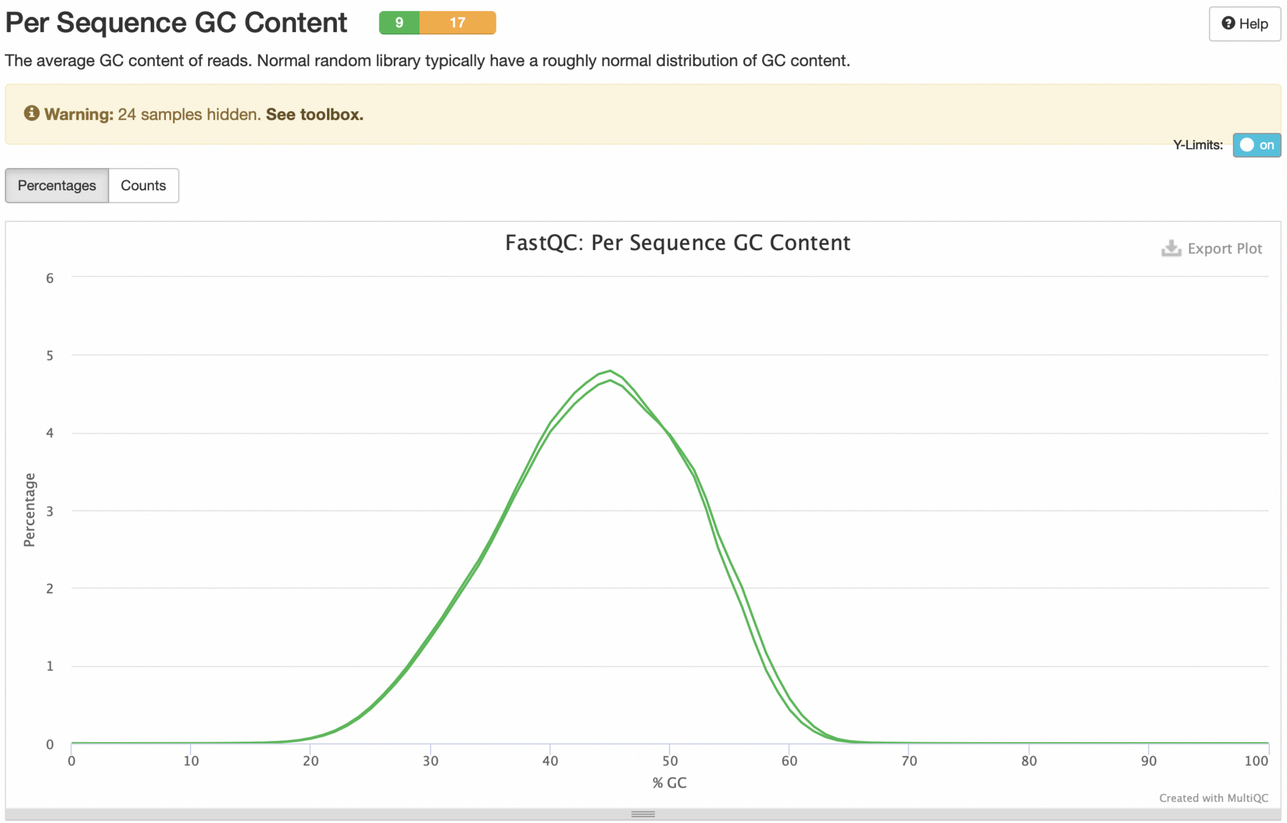
FastQC Per Sequence GC Content



FastQC Example Reports: Good Illumina Data

Per Sequence GC Content

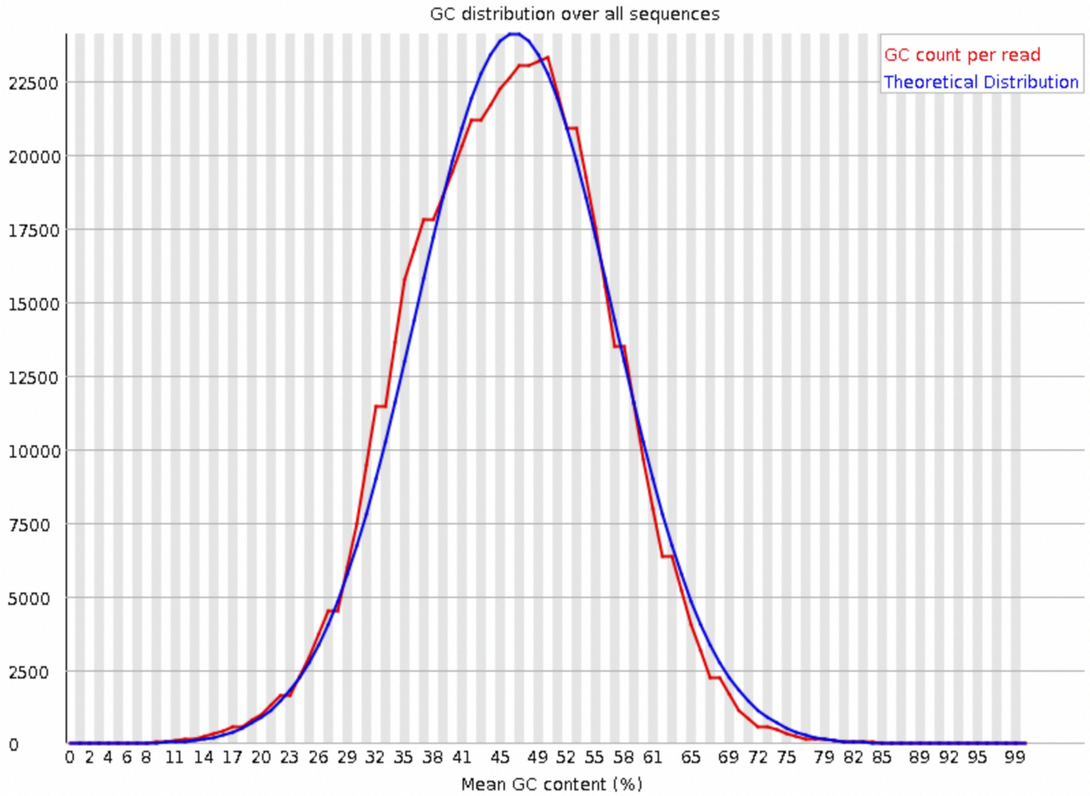




FastQC Per Sequence GC Content

Good result

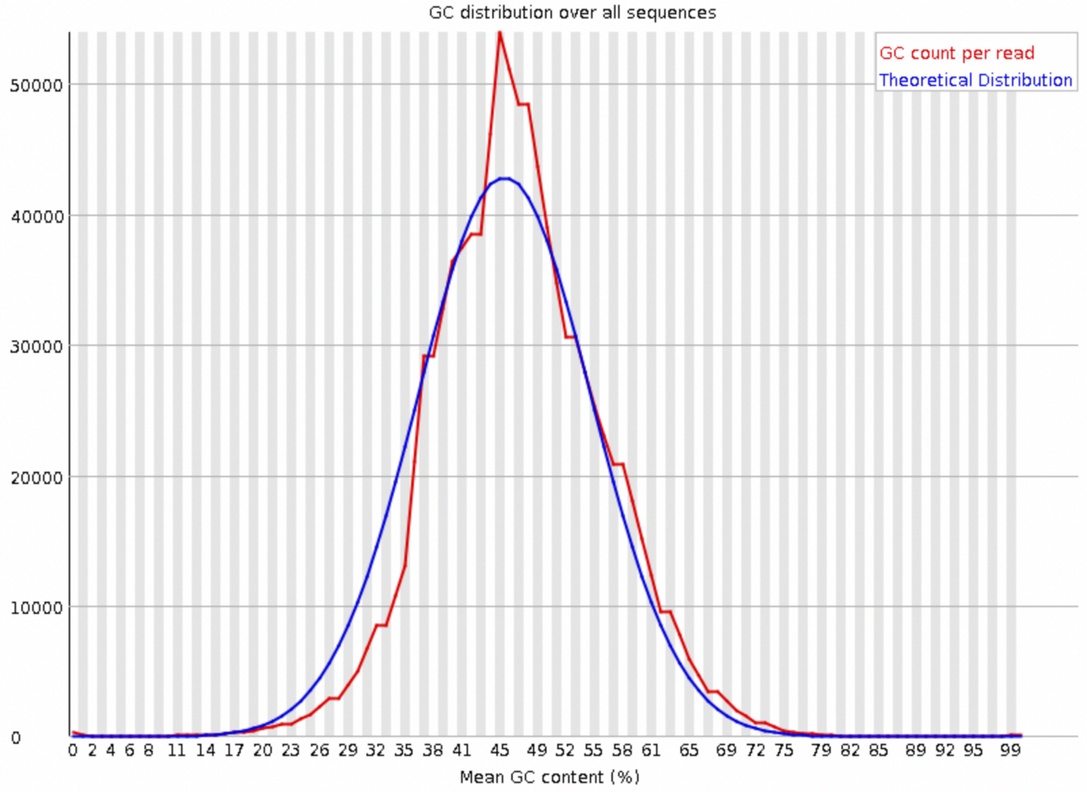
Per sequence GC content



FastQC Example Reports: Good Illumina Data

Bad result

Per sequence GC content



FastQC Example Reports: Bad Illumina Data

FastQC Overrepresented Sequences

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTT	6157956	10.298229526365521	Clontech SMART CDS Primer II A (100% over 26bp)
AGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTT	714216	1.1944158580221549	Clontech SMART CDS Primer II A (100% over 25bp)
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	544134	0.9099800039330219	No Hit
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	104346	0.1745025554190606	No Hit

FastQC Overrepresented Sequences

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTT	6157956	10.298229526365521	Clontech SMART CDS Primer II A (100% over 26bp)
AGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTT	714216	1.1944158580221549	Clontech SMART CDS Primer II A (100% over 25bp)
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	544134	0.9099800039330219	No Hit
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	104346	0.1745025554190606	No Hit

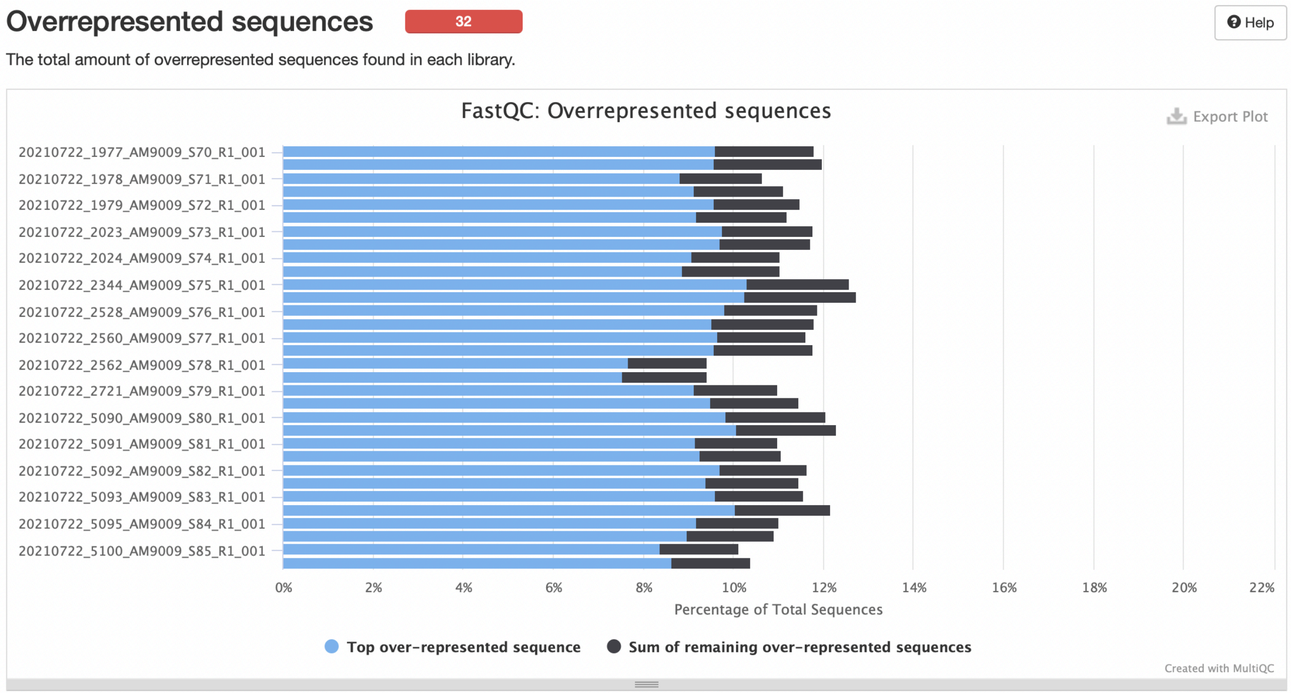
Solution: It depends on the source of the overrepressented sequence:

- **Primers/Adapters:** Trim them an re-run FastQC.
- **No Hit:**
  - **polyA/T tails (in RNA-seq):** Ignore them, they won't align since they are a post-transcriptional modifications.
  - **Other:** Possible contamination (other organism, rRNA in RNA-seq,...). Remove the undesired reads.

Overrepresented Sequences

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTT	6157956	10.298229526365521	Clontech SMART CDS Primer II A (100% over 26bp)
AGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTT	714216	1.1944158580221549	Clontech SMART CDS Primer II A (100% over 25bp)
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	544134	0.9099800039330219	No Hit
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	104346	0.1745025554190606	No Hit



FastQC Overrepresented Sequences

Overrepresented sequences

No overrepresented sequences

Good result

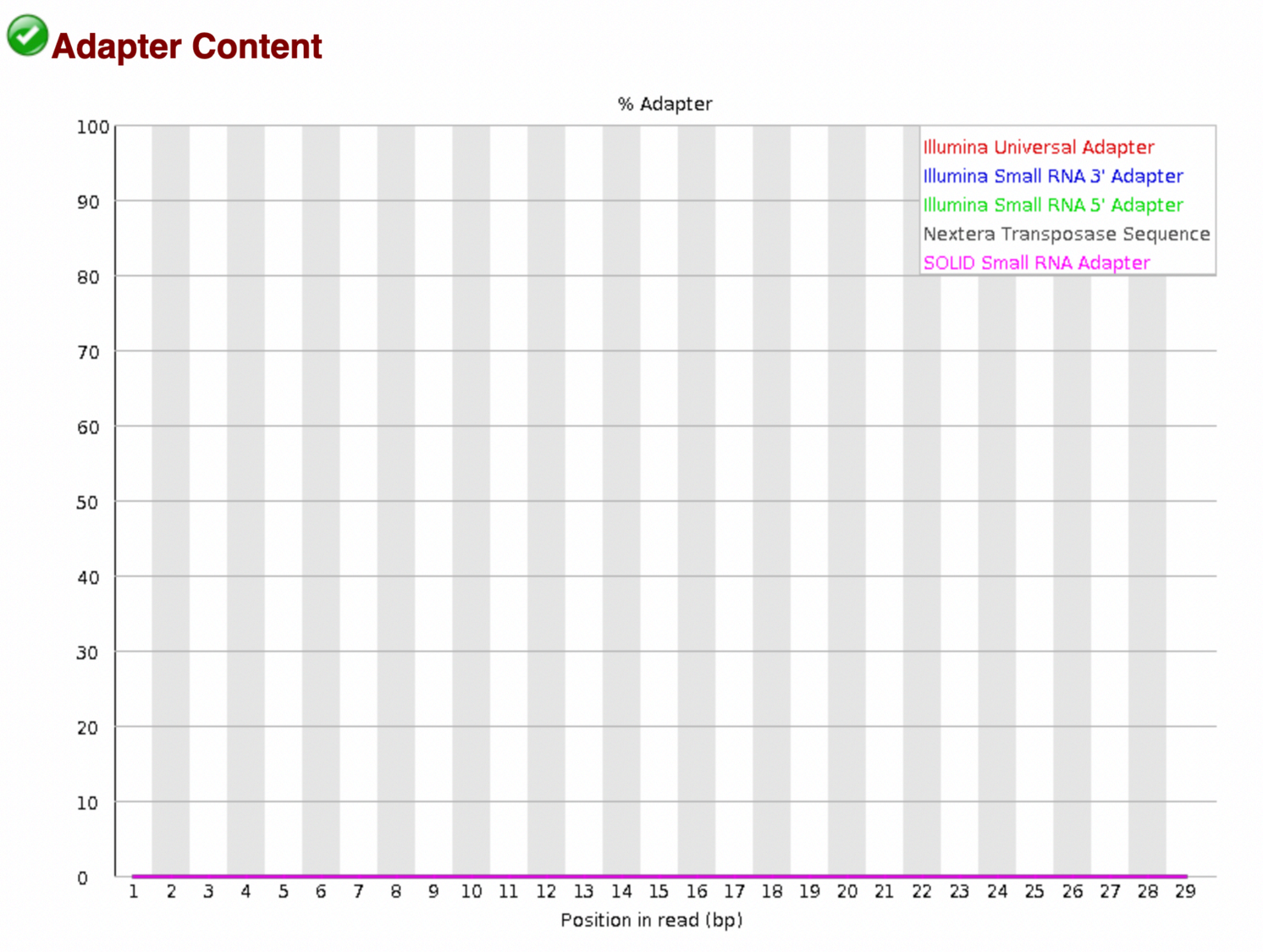
Bad result

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
AGAGTTTTATCGCTTCCATGACGCAGAAGTTAACACTTTC	2065	0.5224039181558763	No Hit
GATTGGCGTATCCAACCTGCAGAGTTTTATCGCTTCCATG	2047	0.5178502762542754	No Hit
ATTGGCGTATCCAACCTGCAGAGTTTTATCGCTTCCATGA	2014	0.5095019327680071	No Hit
CGATAAAAATGATTGGCGTATCCAACCTGCAGAGTTTTAT	1913	0.4839509420979134	No Hit
GTATCCAACCTGCAGAGTTTTATCGCTTCCATGACGCAGA	1879	0.47534961850600066	No Hit
AAAAATGATTGGCGTATCCAACCTGCAGAGTTTTATCGCT	1846	0.4670012750197325	No Hit
TGATTGGCGTATCCAACCTGCAGAGTTTTATCGCTTCCAT	1841	0.46573637449150995	No Hit
AACCTGCAGAGTTTTATCGCTTCCATGACGCAGAAGTTAA	1836	0.46447147396328753	No Hit
GATAAAAATGATTGGCGTATCCAACCTGCAGAGTTTTATC	1831	0.4632065734350651	No Hit
AAATGATTGGCGTATCCAACCTGCAGAGTTTTATCGCTTC	1779	0.45005160794155147	No Hit

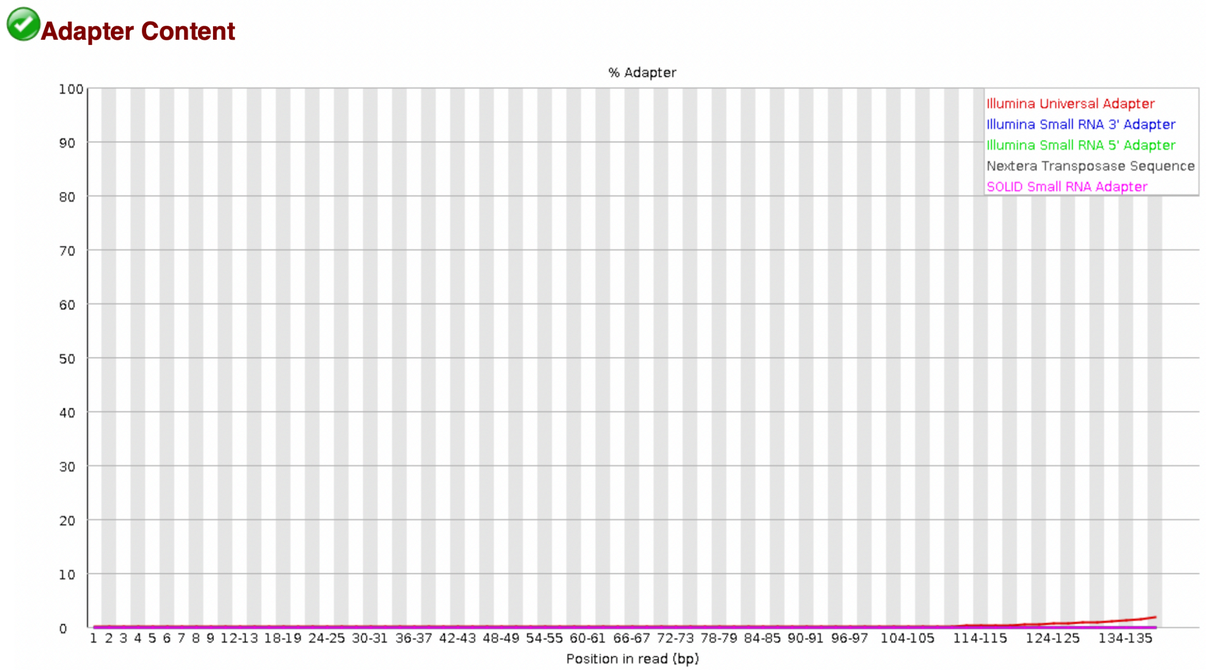
FastQC Example Reports: Bad Illumina Data

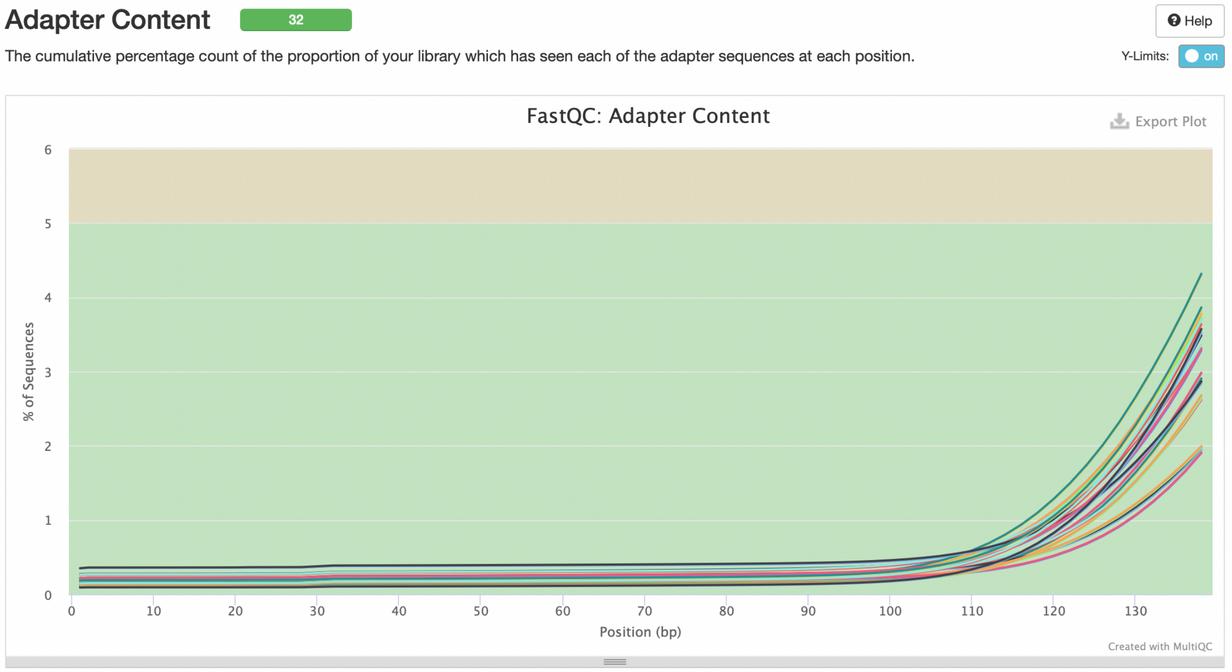
FastQC Adapter Content



FastQC Example Reports: Good Illumina Data

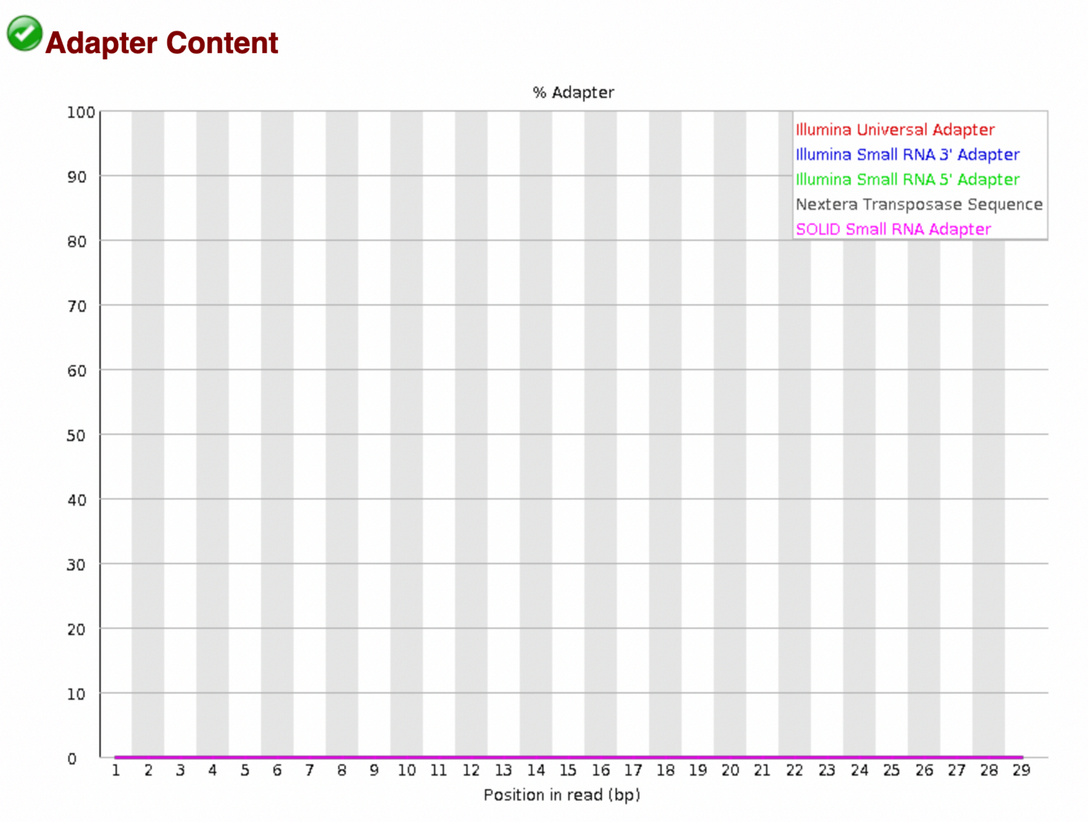
Sequence Adapter Content





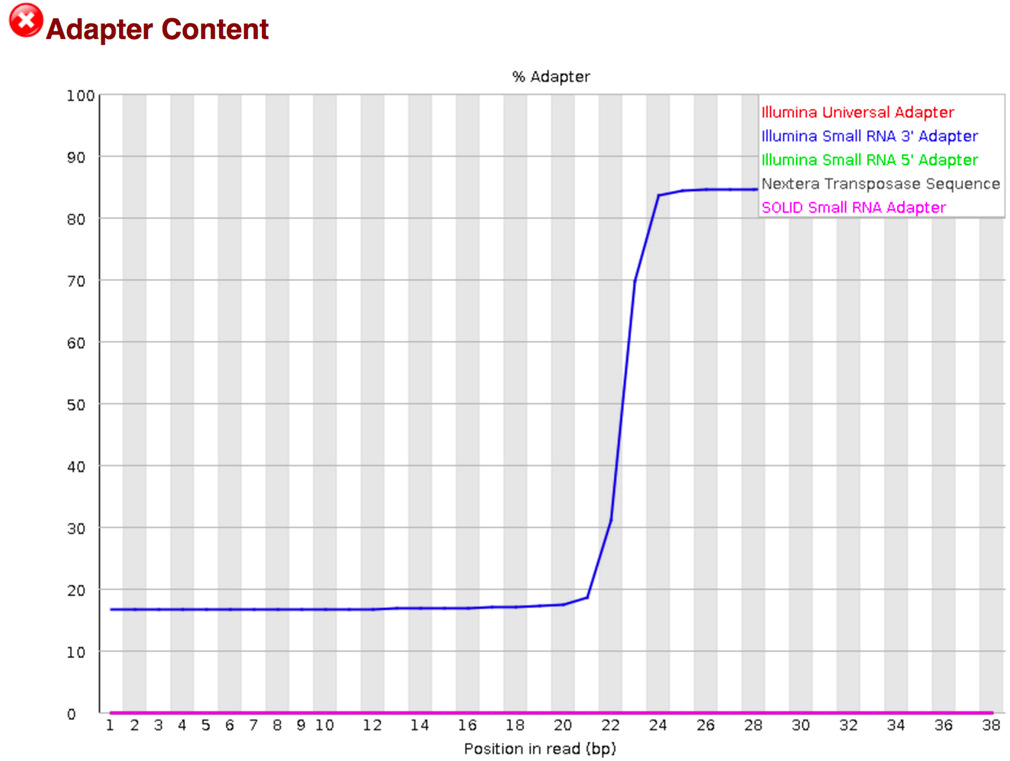
FastQC Adapter Content

Good result



FastQC Example Reports: Good Illumina Data

Bad result



Biostars post #258230

Solution: Trim the adapters.

Further Info

You can check more information about these plots and examples of good and bad data at www.bioinformatics.babraham.ac.uk/projects/fastqc

Exercise

QC of the OVCA case

Study Case - OVCA

Tumor type: Patient with Ovarian Cancer

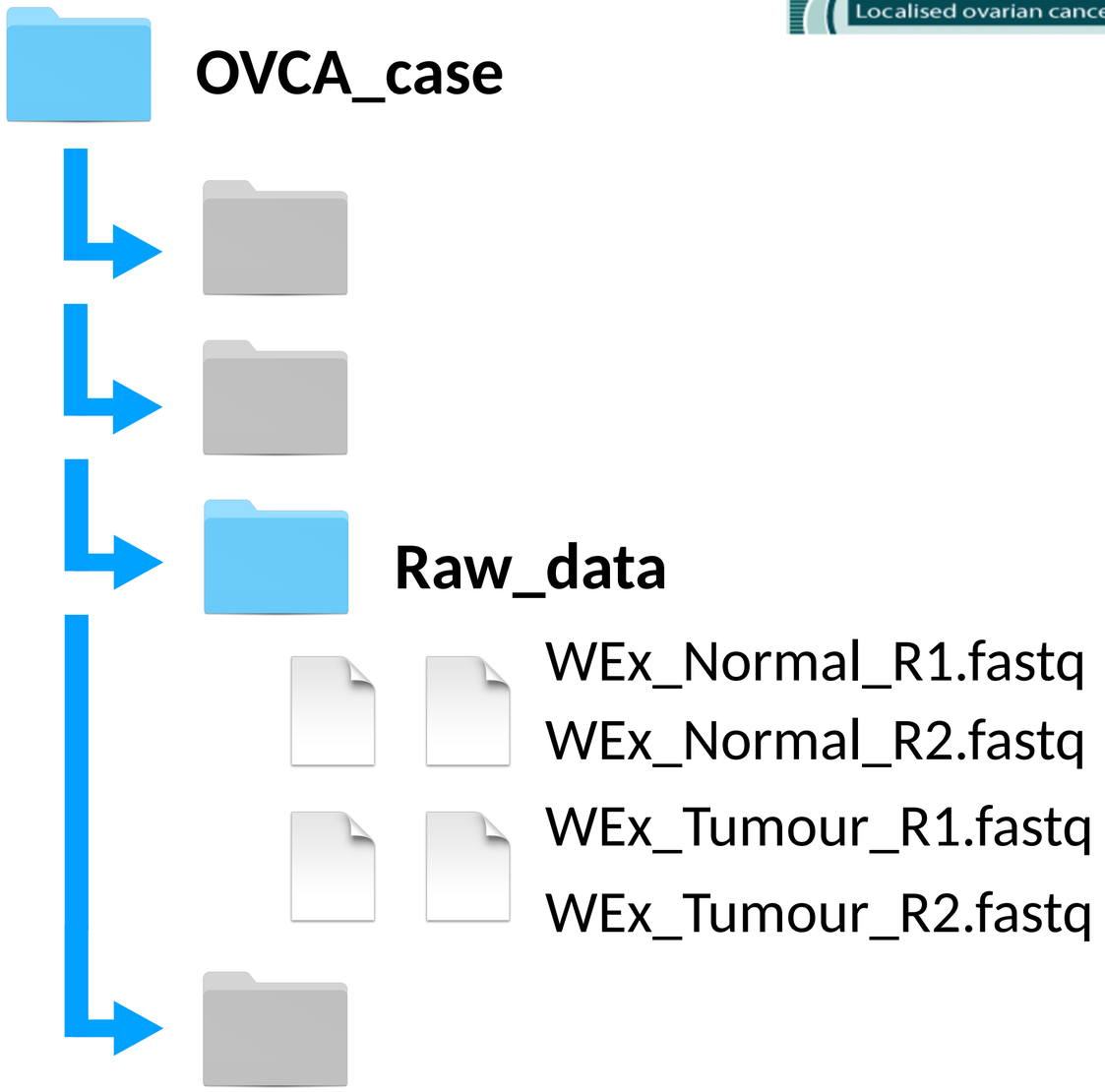
Sequencing platform: Illumina HiSeq2000

Type of data: Whole Exome Sequencing

Samples: 2 samples of tumor with matched healthy tissue (epithelium)

Files: download link here

NOTE: This data was simulated and reduced



Uncompress the data

1. Download the data and save it to your Desktop

2. Open a terminal (Ctrl + Alt + T) and move to the Desktop

```
$ cd /home/$USER/Desktop
```

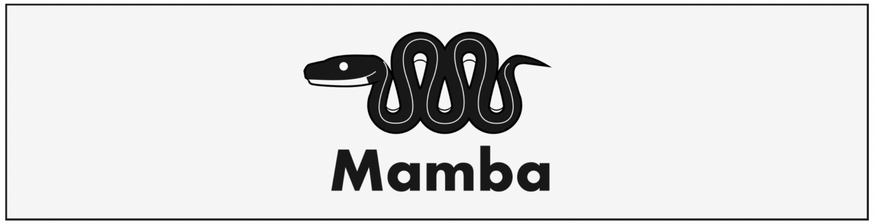
3. Untar the folder

```
$ tar xvf OVCA_case.tar
```

Install FastQC and MultiQC

1. Install FastQC and MultiQC through mamba

```
$ conda install -n base -c conda-forge mamba
$ mamba create -n QC -c bioconda fastqc multiqc
```



https://github.com/mamba-org/mamba

Mamba is a reimplementation of conda written in C++.

It uses the same syntax as conda and works much faster, so it allows us to download the programs really quick.

Execution

1. Activate the conda environment

```
$ conda activate QC
```

2. Move to Raw_data folder

```
$ cd OVCA_case/Raw_data
```

3. Exercute FastQC

```
$ fastqc *
```

4. Execute MultiQC

```
$ multiqc *
```

Execution

5. Deactivate the environment

```
$ conda deactivate
```

IMPORTANT: FastQC and MultiQC are executed automatically in varca, as we'll see tomorrow.

Questions

10 min

- **What sequence depth was run in the experiment? (# of sequenced reads) What is the read length?**
- **What Phred Score encoding is detected by the algorithm?**
- **How is the general quality of each file?**
- **Is there any plot with an error/warning? Which one(s)? Any ideas why?**

Answers

- **Sequence depth and length:** 1.4 M reads of 75 bp length.
- **Phred Score encoding:** Sanger/Illumina 1.9.
- **Overall quality:** Quite good.
- **Warning/errors in** GC content and Overrepressented sequences (polyNs). There are a lot of N nucleotides because this is simulated data created on purpose to raise errors/ warnings.