

\* Note: **Rfastp** is used for trimming.

1. From the dropdown on the left hand panel, select the sample to run trimming.

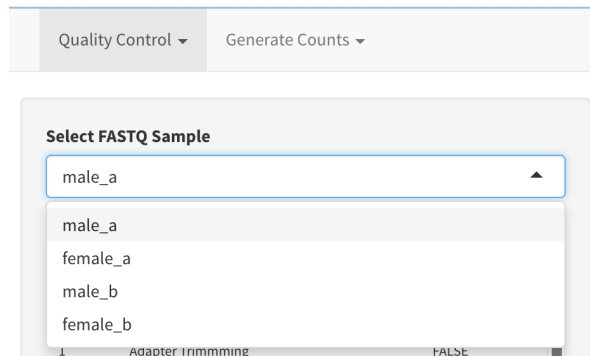


Fig 3.1: Select sample to trim

2. General guidelines when configuring trim settings
  - a. Refer below for some examples on how to use fastqc plots to configure trim settings.

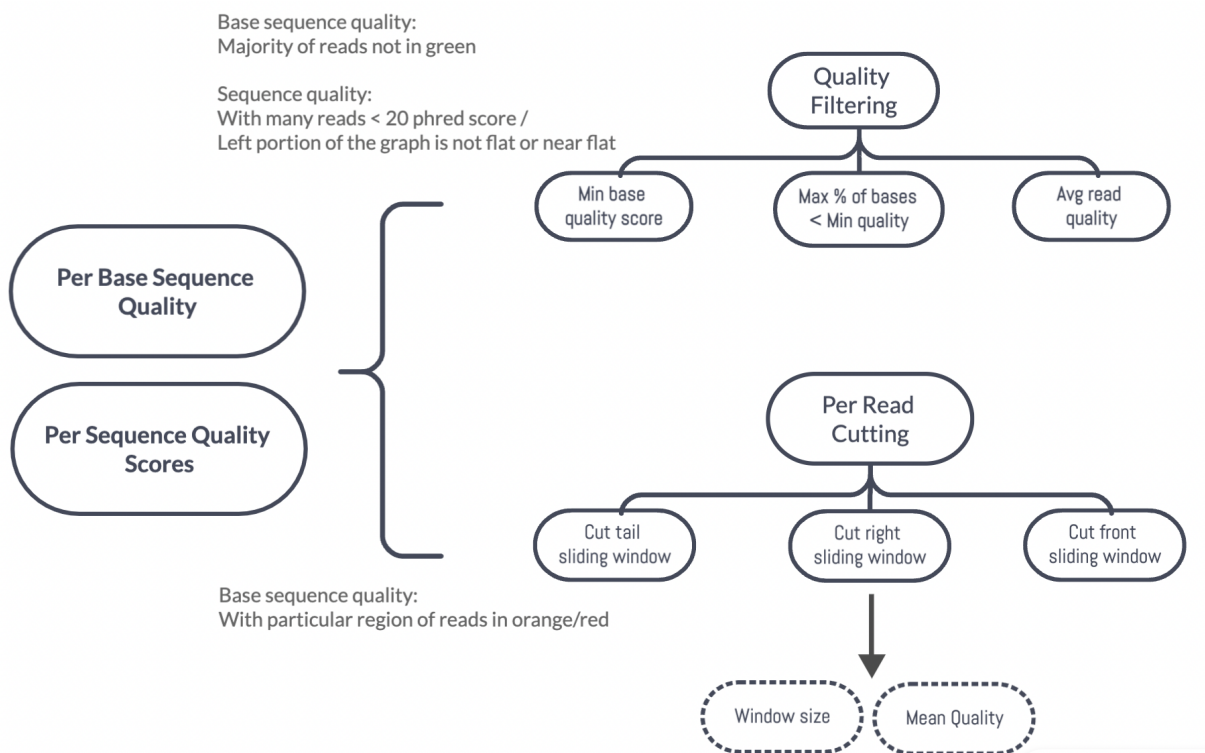


Fig 3.2: Use Per Base Sequence Quality & Per Sequence Quality

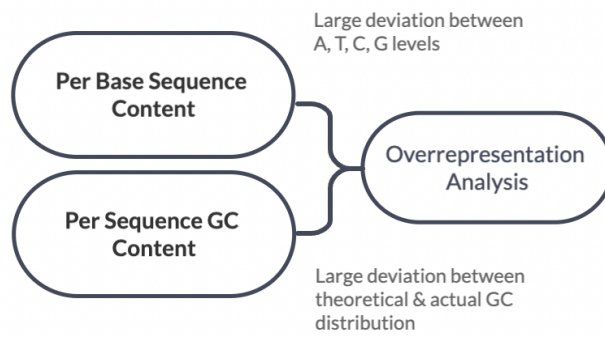


Fig 3.3: Use Per Base Sequence Content & GC Content

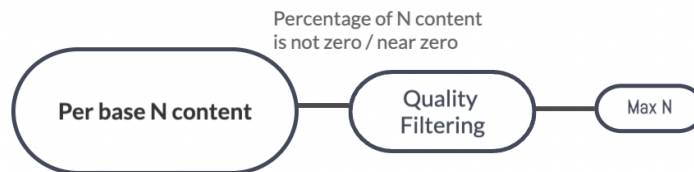


Fig 3.4: Use N content

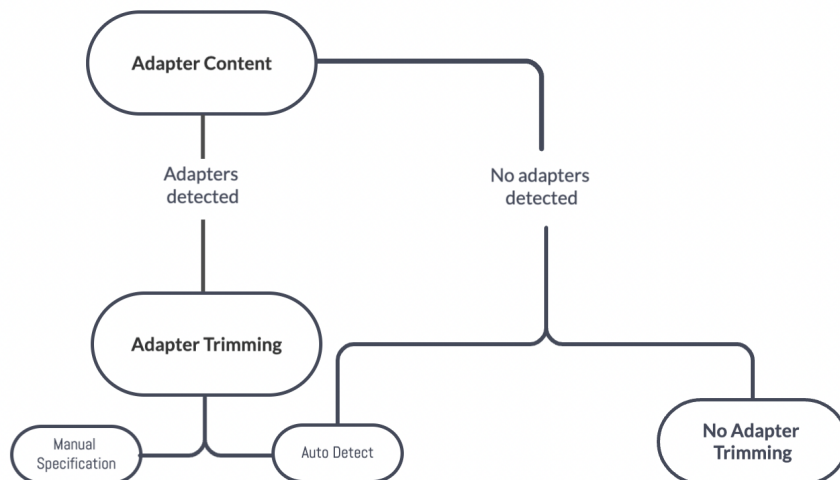


Fig 3.5: Use Adapter Content

- b. After trimming and quality filtering, remember to enable **filtering by length** (as reads may become too short to meet the minimum length requirement by HISAT2 which will fail to align).

**Length**

Filter by length ☒ **Enable**

Note that length filtering is applied as a last step.

**Min read length**

15

Reads shorter than min length will be discarded.

**Max read length**

0

Reads shorter than min length will be discarded. 0 means no limitation.

Fig 3.6: Enable length filtering

- c. Enabling overrepresentation analysis will significantly increase trimming time.
3. All trim configurations are summarized on the left table. Press 'Run Trimming'.

**Fastp Trim Settings**

	setting	value
1	Adapter Trimming	FALSE
2	Quality Filtering	FALSE
3	Max N bases	5
4	Cut low quality tail	FALSE
5	Cut low quality right	FALSE
6	Cut low quality front	FALSE
7	Length Filtering	FALSE
8	Trim front(R1)	0
9	Trim tail(R1)	0
10	Trim to length(R1)	0
11	Trim polyG tail	FALSE

**Run Trimming** ← Click once done

Fig 3.7: View setting and run trimming

4. Once trimming for the sample is completed, select the fastq file from the dropdown to view results.  
(note: Dropdown only contains trimmed fastq json reports from sample selected for trimming)

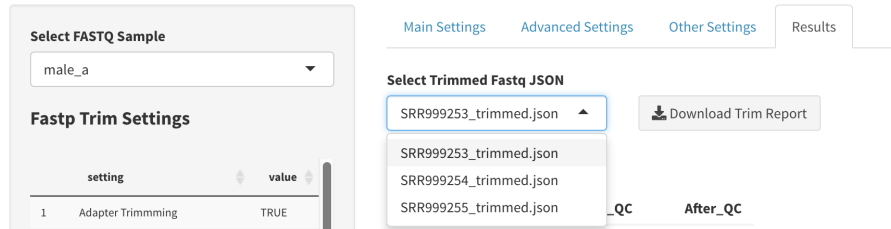


Fig 3.8: Select trimmed fastq file

5. Different Rfastp trim configurations can be set for each sample and the same sample can be trimmed again with different settings (with original fastq files uploaded, **not** the trimmed fastq files afterwards).
6. Results
  - a. Click the “Download Trim Report” button beside the dropdown to view a Rfastp HTML report for the fastq file selected.
  - b. Scroll further down to view comparison plots before and after the fastq file selected was trimmed by Rfastp.
  - c. In both the html report and plots, overrepresented sequences will only be shown if overrepresentation analysis was enabled for the sample.

Example report with overrepresented sequences:

[https://github.com/paigerollex/gene\\_cloud\\_omics/blob/main/output\\_data/trim\\_report\\_ovr.pdf](https://github.com/paigerollex/gene_cloud_omics/blob/main/output_data/trim_report_ovr.pdf)

Example report without overrepresented sequences:

[https://github.com/paigerollex/gene\\_cloud\\_omics/blob/main/output\\_data/trim\\_report\\_no\\_ovr.pdf](https://github.com/paigerollex/gene_cloud_omics/blob/main/output_data/trim_report_no_ovr.pdf)

Summary:

	Before_QC	After_QC
total_reads	10337265.00	10308967.00
total_bases	785632140.00	738865720.00
q20_bases	757805034.00	726768951.00
q30_bases	710828064.00	682365304.00
q20_rate	0.96	0.98
q30_rate	0.90	0.92
read1_mean_length	76.00	71.00
gc_content	0.46	0.45

Fig 3.9: Rfastp summary

Base Quality Comparison:

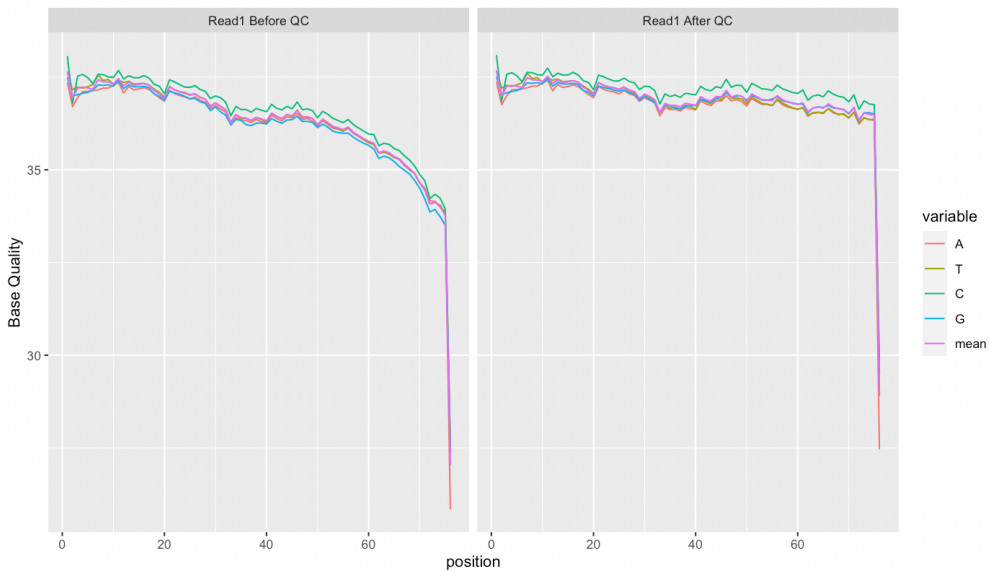


Fig 3.10: Per Sequence Quality Scores (Before & After)

Base Content Comparison:

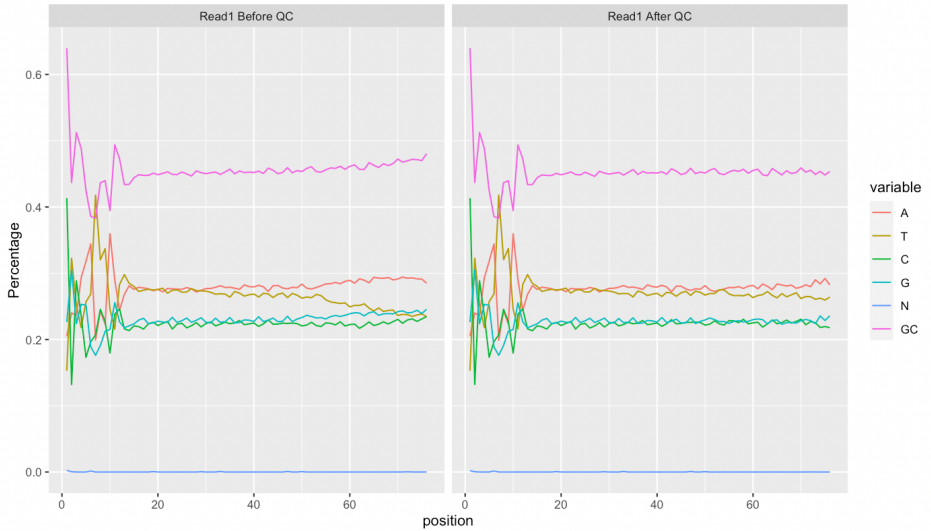


Fig 3.11: Per Base Sequence Content (Before & After)

Over-Represented Sequences:

Show 

10

 entries

Search:

	Count
AAAAAAGATTGCGACCTCGATGTTGGATTAAGATATAATT	158
AAAAAATTAAGTTACTTTAGGGATAACAGCGTAATTTTTT	184
AAAAACATGCTTTTTGAATTATATATAAAGTCTAACCTG	58
AAAAAGATTGCGACCTCGATGTTGGATTAAGATATAATTT	31
AAAAATAATATTCGCCTGTTTAACAAAAACATGCTTTTTT	14
AAAAATTAAGTTACTTTAGGGATAACAGCGTAATTTTTTTT	24
AAAACATGCTTTTTGAATTATATATAAAGTCTAACCTGC	23
AAAAGATTGCGACCTCGATGTTGGATTAAGATATAATTTT	61
AAAATAATATTCGCCTGTTTAACAAAAACATGCTTTTTTG	7
AAAATTAAGTTACTTTAGGGATAACAGCGTAATTTTTTTTG	10

Showing 1 to 10 of 428 entries

Previous

1

2345...43Next

Fig 3.12: Overrepresented Sequences after trimming.