

# RNA-Seq Analysis: QC

Before the alignment, quantification and farther tests we need to evaluate the quality of the sequencing experiment to see if there is any technical problem that could influence the analysis.

**FastQC:** tool to explore the quality of sequencing experiments.

FastQC Read Quality reports (Galaxy Version 0.72+galaxy1)

Short read data from your current history

18: NIKD Raw Data

Select the Dataset Collection with the Single-End reads in this case

Contaminant list

Nothing selected

Adapter list

Nothing selected

If you have a list of sequences that might be contaminants can be added here and the tool will search for its presence

Submodule and Limit specifying file

Nothing selected

Same as contaminant list but for adapters. The tool will look for adapters and report its presence

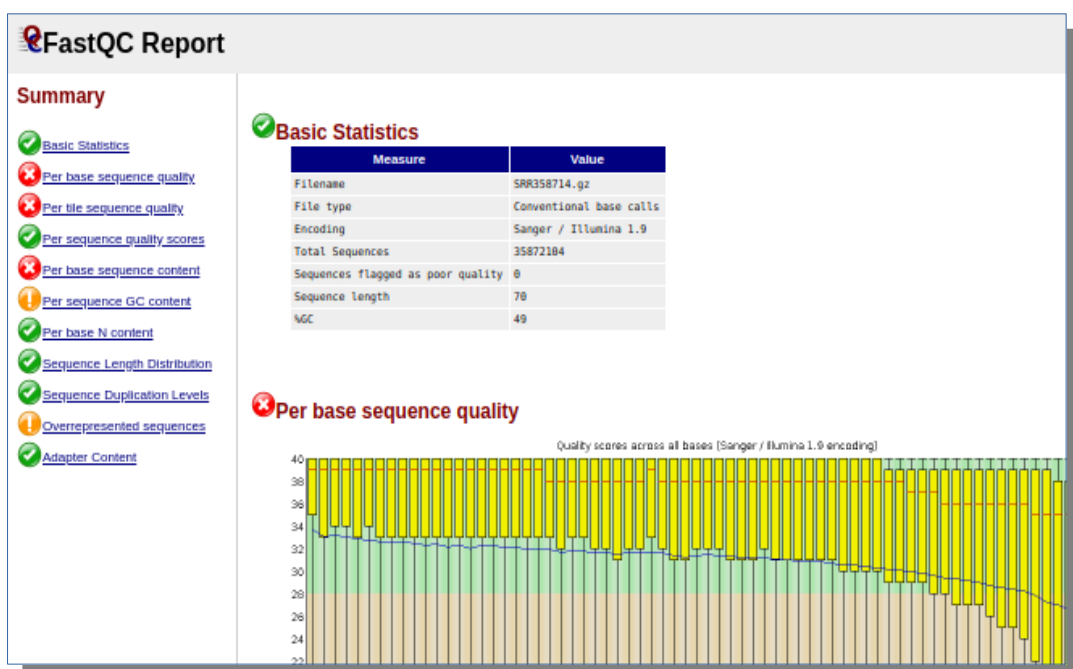
Lower limit on the length of the sequence to be shown in the report

length of Kmer to look for

7

Execute

- We can leave all the options as default
- Repeat the step for both collections of Single-End reads, the WT and the KO
- The tool produces an html report with all the metrics.
- Explore the output and compare it with the FastQC we will do after processing the raw reads



# RNA-Seq Analysis: Preprocess Reads

This step will help to remove low quality reads and remove adapter contaminations. This will improve the later alignments and quantification.

**Trim-Galore:** Will preprocess the reads

Trim Galore! Quality and adapter trimmer of reads (Galaxy Version 0.6.3)

☆ Favorite

Versions

▼ Options

Is this library paired- or single-end?

Single-end

Reads in FASTQ format

18: Nr1KO Raw Data

This is a batch mode input field. Separate jobs will be triggered for each dataset selection.

Adapter sequence to be trimmed

Automatic detection

Remove N bp from the 3' end

Instructions: Instructs Trim Galore! to remove N bp from the 3' end of read 1 after adapter/quality trimming has been performed. This may remove some unwanted bias from the 3' end that is not directly related to adapter sequence or basecall quality. (-three\_prime\_clip\_R1)

Trim Galore! advanced settings

Use defaults

You can use the default settings or set custom values for any of Trim Galore!'s parameters.

RRBS specific settings

Use defaults (no RRBS)

Email notification

Yes No

Send an email notification when the job completes.

✓ Execute

Indicate it is a Single-End sequencing experiment

Select the Dataset Collection with the Single-End reads in this case

- The rest of the options will be OK to leave them as default.
- Repeat the step for both, the WT collection and the KO collection.
- Proceed later with another round of FastQC steps on the processed reads and compare it with the QCs made before. Notice how the quality plots are improved