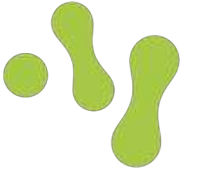


Quality Control of NGS data

Malin Larsson

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FastQ files



```
@HWUSI-EAS100R:6:73:941:1973#0/1  
GATTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT  
+  
!' '*((( (***) ) %%%++) (%%%) .1***-+*' ') **55CCF>>>>>CCCCCCC65
```

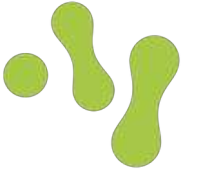
1st row: sequence identifier (machine ID, x-y coordinates, additional info)

2nd row: The actual sequence

3rd row: starts with “+” and optionally the same identifier as in the 1st row

4th row: Quality score for each base in read

Phred Quality Scores



```
+SEQ_ID
!' '*(((('***+))%%%++) (%%%) .1**
```

A quality value Q is an integer representation of the probability p that the corresponding base call is incorrect.

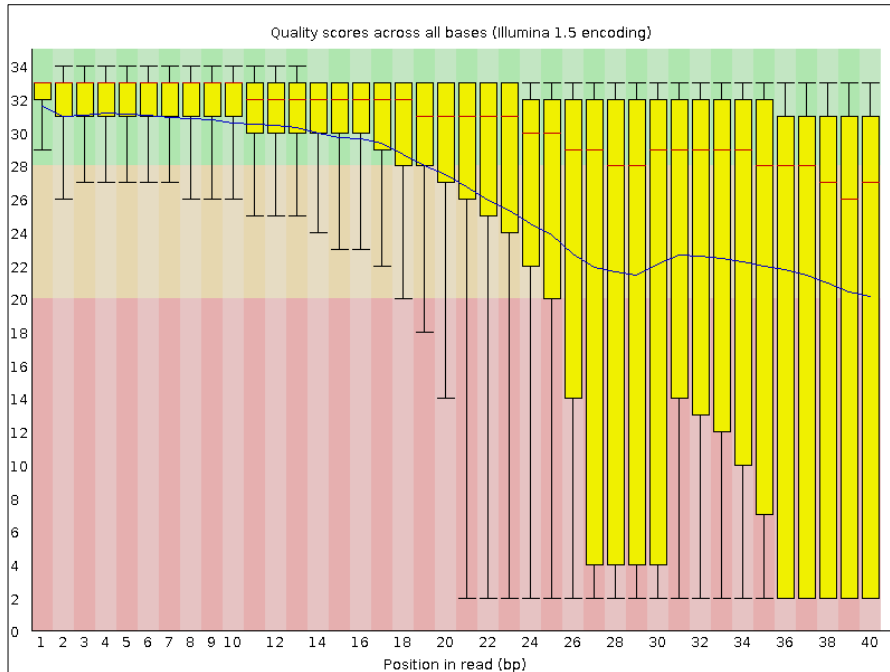
$$Q = -10 \log_{10} P \quad \longrightarrow \quad P = 10^{\frac{-Q}{10}}$$

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99.99%
50	1 in 100000	99.999%

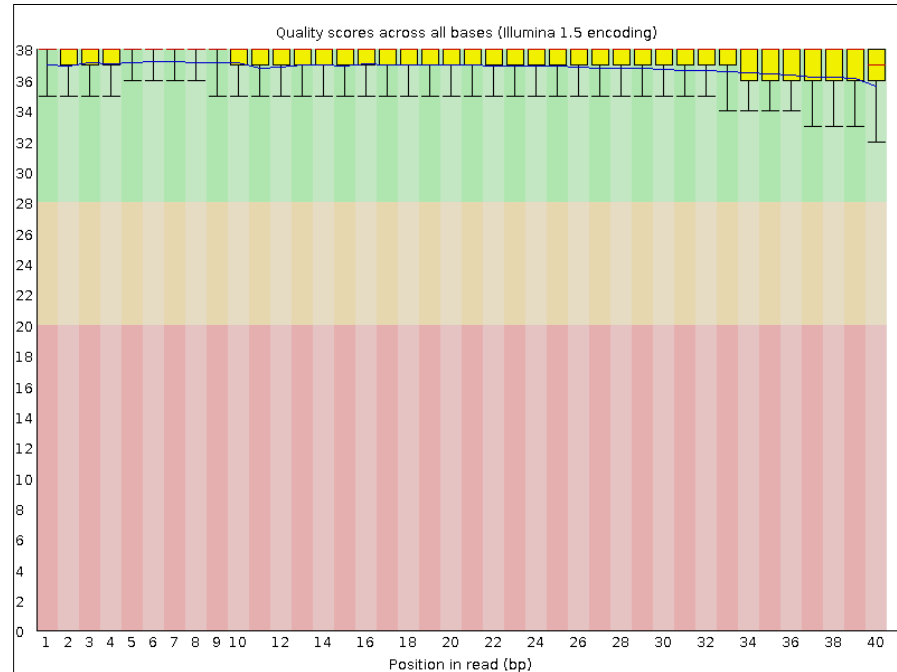


FastQC

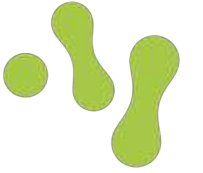
Bad qualities:



Good qualities:

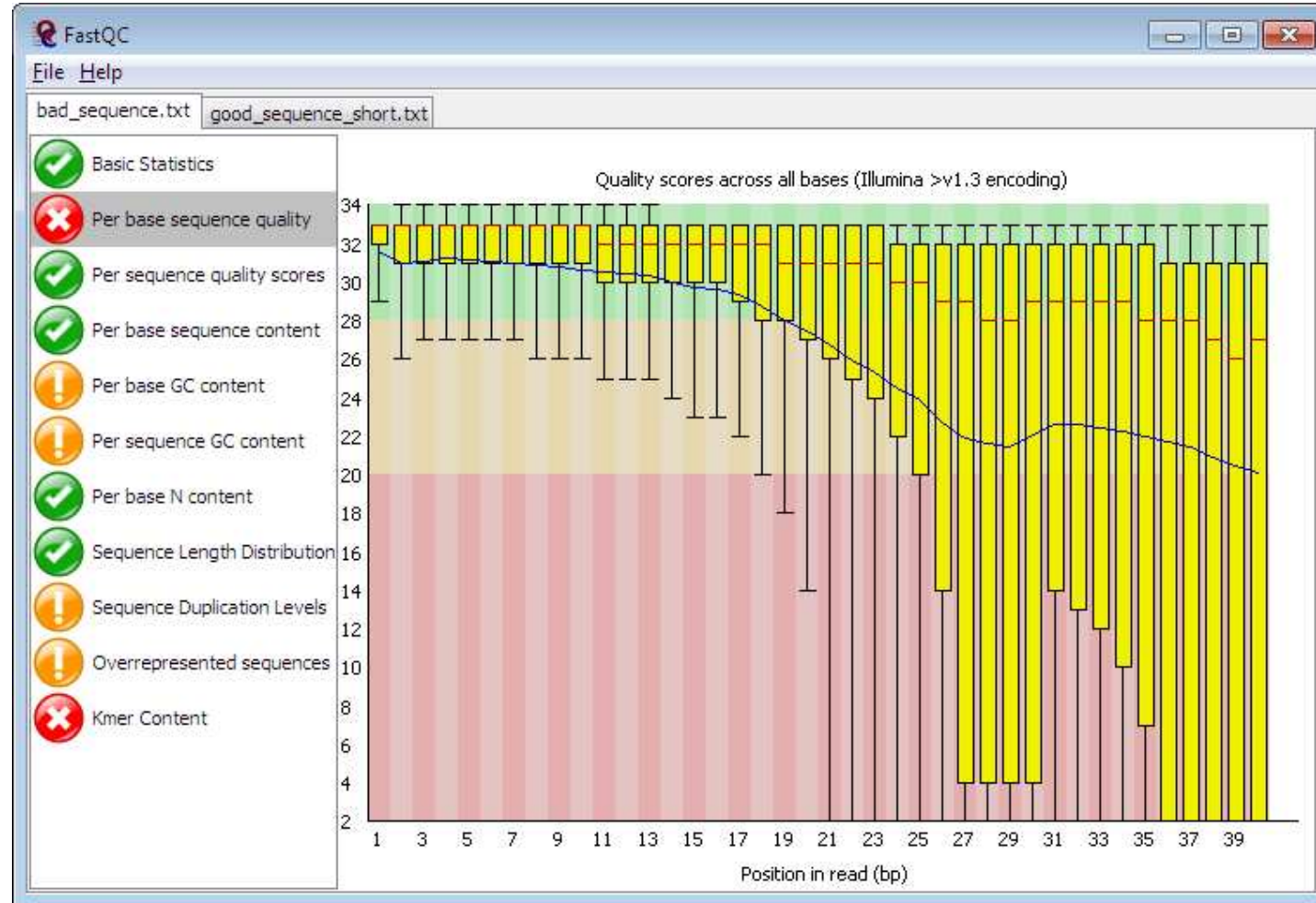
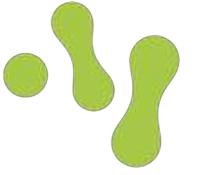


What is QC?

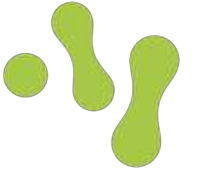


- Different NGS application have their own problem areas and requires their own QC strategy
- Today: Focus on QC for whole genome sequencing
- For variant calling it is important to look at quality score distribution, sequence length distribution and duplication levels.
- Thursday: More details on QC for RNA-seq

FastQC



FastQC link



<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>