Introduction to quality check and trimming

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Joint Training Course on NGS
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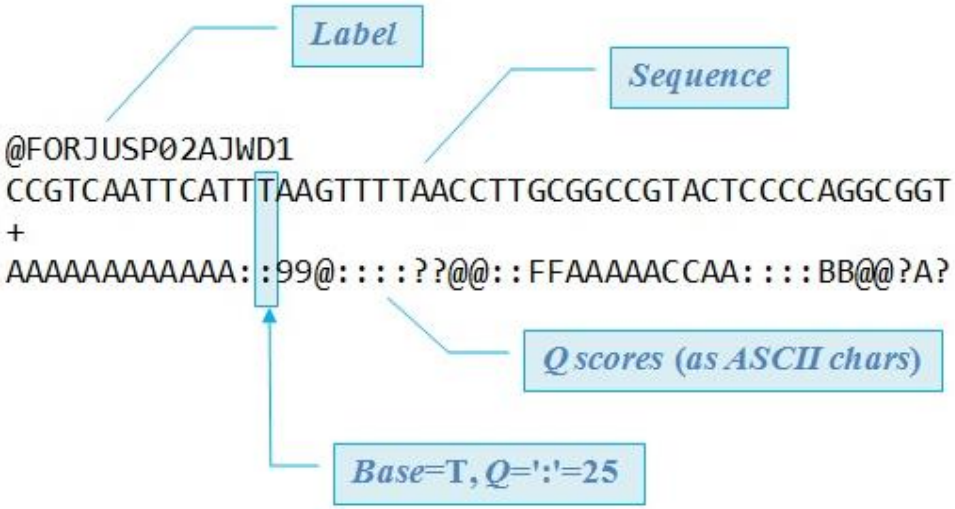
.fastq files



Each .fastq file covering a 5 Mb genome at 50X weights about **500 MB**

Phred quality scores are logarithmically linked to error probabilities

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 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Phred quality score

$$Q = -10 \log_{10} P$$

from 0 to 93 using ASCII characters 33 to 126

.fastq files

@

```
@X1L6C:01561:00672
AAATATCACCAAATAAAAAACGCCTTAGTAAGTATTTTTCAGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTG
GATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTGGTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCAC
TAAATACTTTAACCAATATAGGCATAGCGCACAGACAGATAAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCA
CCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAAGCCCGCA
CCTGACAGTGCGGGCTTTTTTCGACCAAAGGTAACGAGGTAACAACCATGCG
+
CC:9::FBC<CD7:88888(:>><C<CCCC<CCBBAAB/A@A8888,;<@;AABBB=?;B98992:B<
CGBBCGDCC??BCC;BB<ADEEED*CCCAAACCCBCABBDDBB>B??A;999;@8=>199A7>9::CBCH:B:>>>)999)
77037;<7==5=@@BBCC:C@BBB9B<E<D9>?><<6ADCBCBAABB@@@DDCCBA@@==+=.//?B<??AEB::6;DCD>
C:;;;-:9:BC<BBCCC9??<AA;AG<CB>GD@B;;;A<AE;AA<B??@9@C<BB<???BB;BBBAAAA:::BAB099/9>
@========(<<?)99997>>CCEBA>>=>2373333&3:99-33(3--717---43606704/47761
@X1L6C:01104:03031
AGAAGCTGCTATCAGACACTCTTTTTTTAATCCACACAGAGACATATTGCCCGTTGCAGTCAGAATGAAAAGCTGAAAAATA
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TAAAAGCCCCAGGCG
+
@AC=BCCC???B?@@CBB@???>>>>>*?8??>DAABEBCBABCAAA:@@>+9:8>;<;//.
98283988*44449;;9/88:?29:>>5;78333333&399298:6/./DCDDCC';>:ACBDAABB??9::+9<
1444@:?77-3<03368:8755888;:9833)3777'--'--
@X1L6C:03659:02717
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GCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTA
CCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGACCCGCCACTGACCAGTGCG
+
???9?BB@<CAA;A8@?:?@@5::BCCCEC;C=CCC8CEJ8DE;AACF>CC?DDCCCBB:B@???9?;B=B=CAA@?;?BCG
CCCCCCBABBBBCCDDAA2:4;@???CAB@AAA9@@AB?C:;;C;CDCCC>ECCAA<AC<CB>DC<AB=CD=C9::A4::>
CC;@@@A?CI@DDAFKDDD:A@CBCDC::::99199+8;4746@CA?)<444/3:4934333-3888//
@X1L6C:02011:02071
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CAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGTGACGCGTACAG
GAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTCGACCAAAGTAACG
+
=0>>>19;;,;;7=CCDADC;?::::::,5;;==4>273:<@BBCF=CDH;@;MMFEED@?>>>:::::*5/55<
;::@:;:BC=BCBB<B@@@D<@@B:;3:::9@<BB=BD=AC;@B:??3::CAC=CD;;;=BBAB>CC;AA;BAAAA9AD@>>
>>?955>4?9499985555558&4<>2:;661499888...88/56666666$;6/.5:8(..+'++
@X1L6C:01333:03005
GCAATGCCAGGCAGGGCATGTACAGCTACGTACGTCTGAGCATCGATCGATGTACAGCTACGTACGTCTGAGCATCGATCGA
TGTACAGCTACGTACGTCTGAGCATCGATCGATGTACAGCTACG
+
555/55/(///(////(/8:9:<=>?<?@:98A??676<:;;@:5555555554444;=4443333;383338<68>>
68=333118311111111113933644588?==<76992---2+++0/
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@

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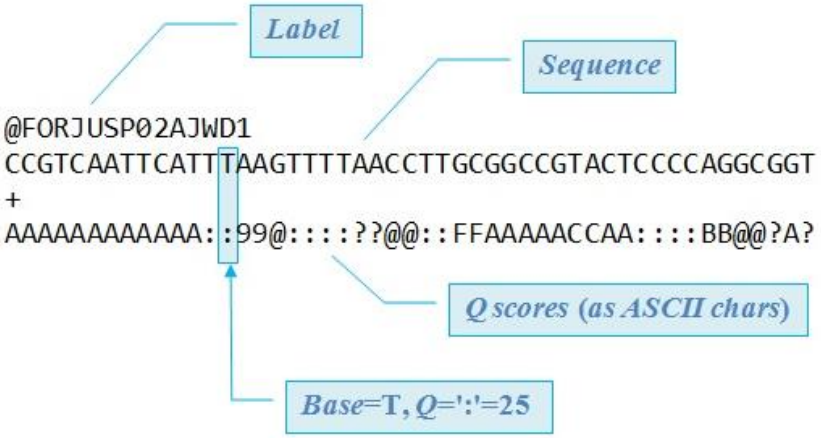
@

@

…and so on

Quality check

Output of NGS sequencers



.fastq file

Input for quality check

Sequencing errors would impact every following application

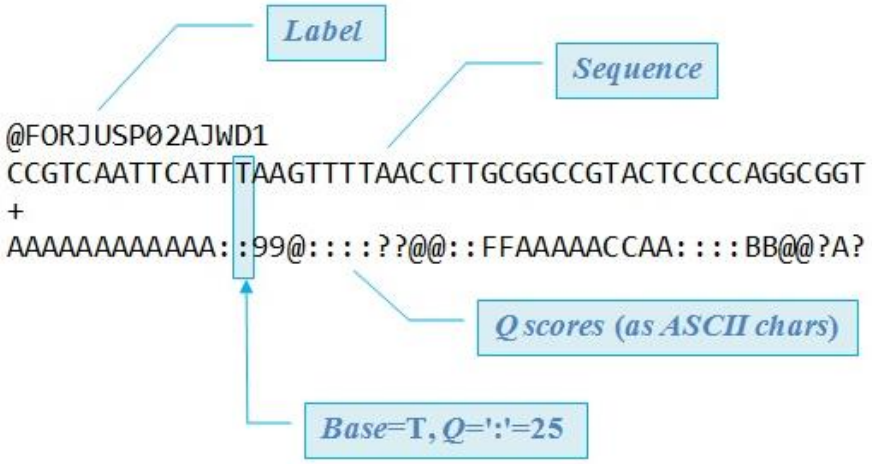
Unreliability of following results (and difficulty to detect the existence of problems!)

Parameters to control

- Phred score
- GC content distribution over all sequences
- Distribution of nucleotides
- Length of the reads
- Coverage

Adoption of corrective actions is possible to minimize these problems

What should be trimmed out?



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- Adaptors and barcodes
- Low quality positions
- Very short sequencing reads
  (only for Ion Torrent reads)

What should be trimmed out?

Eg: Ion Torrent reads

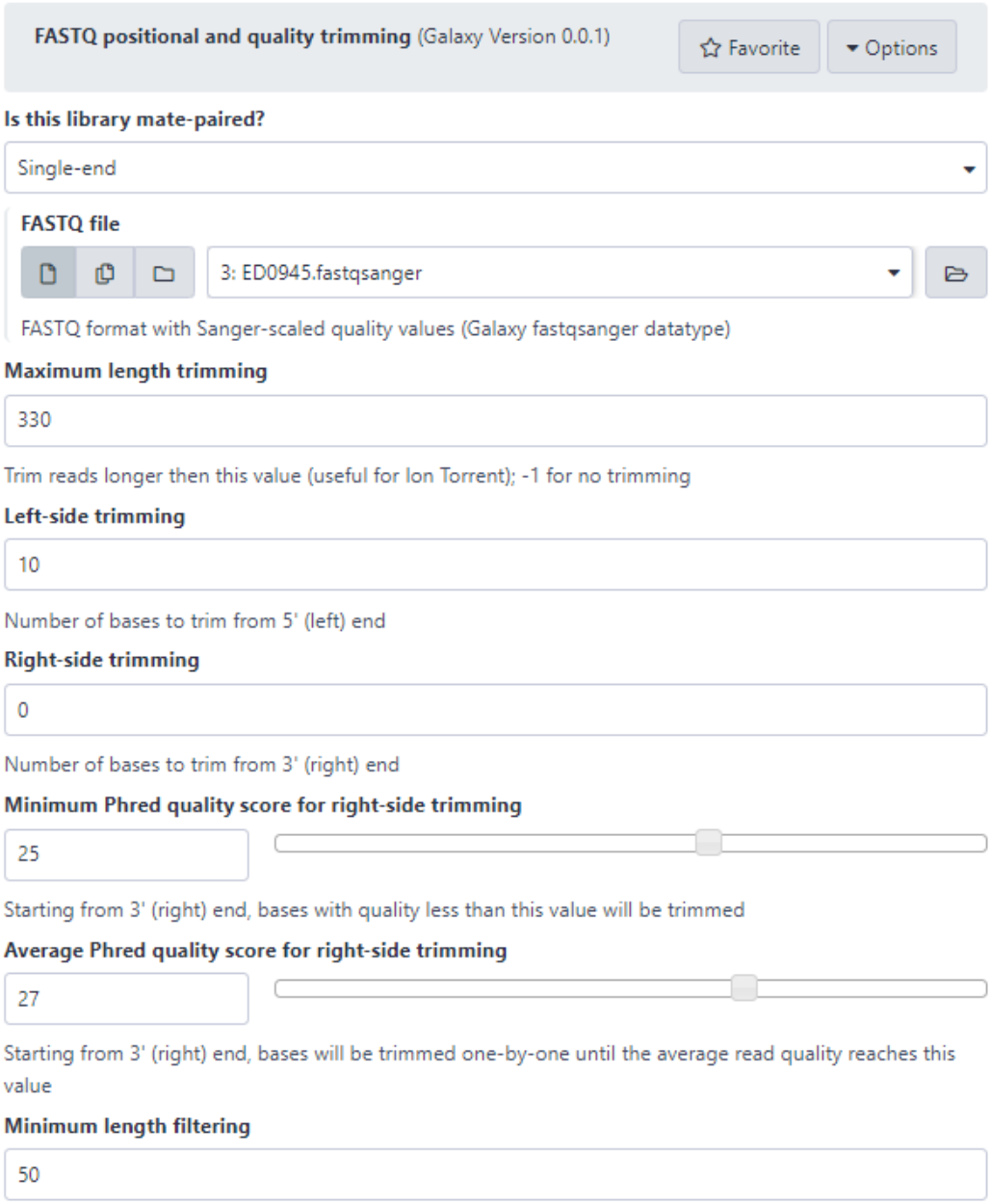
Maximum length trimming

Left-side trimming

Minimum Phred quality score for right-side trimming

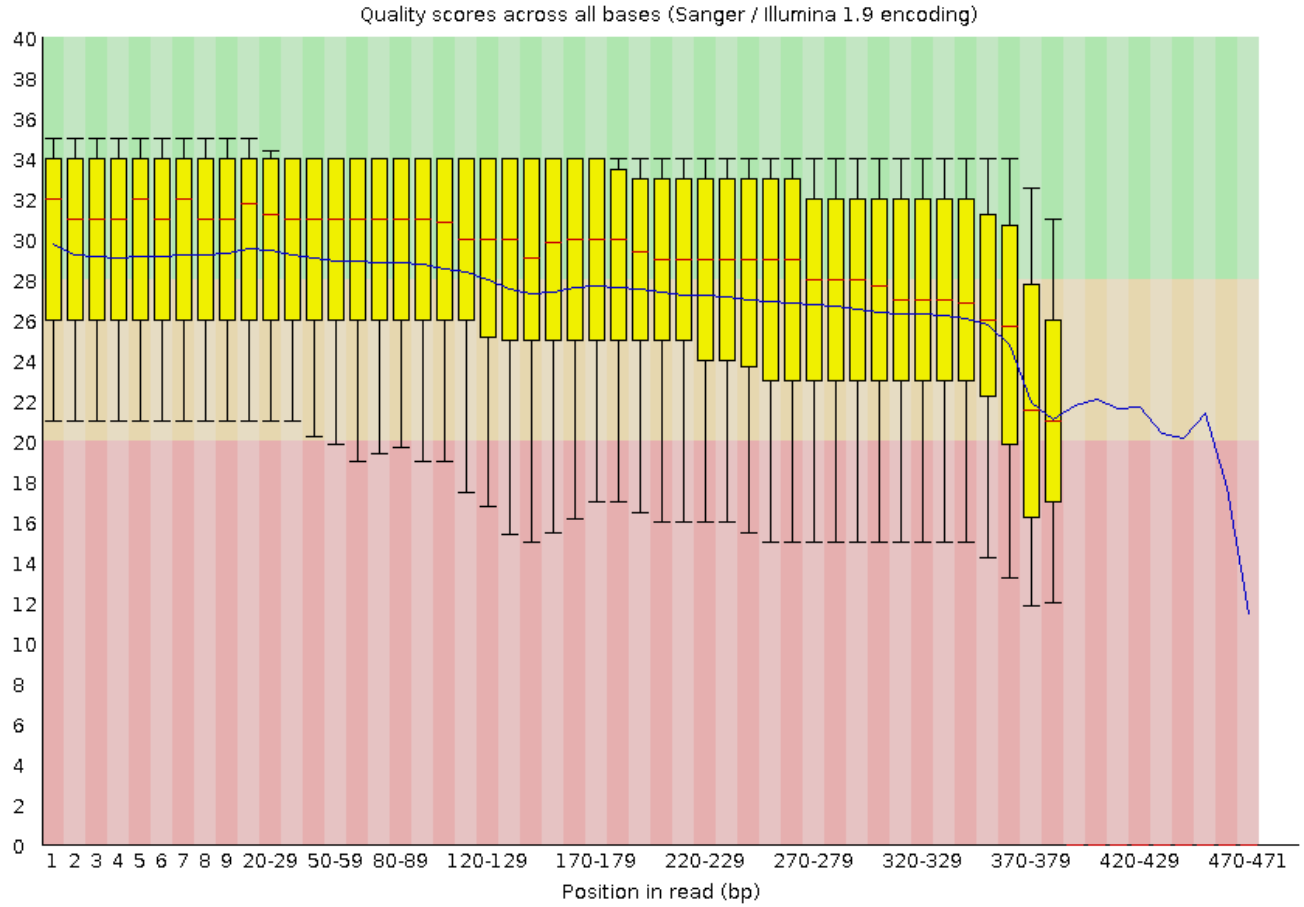
Average Phred quality score for right-side trimming

Minimum length filtering



FastQC – quality check of raw data

Per base sequence quality



The central red line is the median value

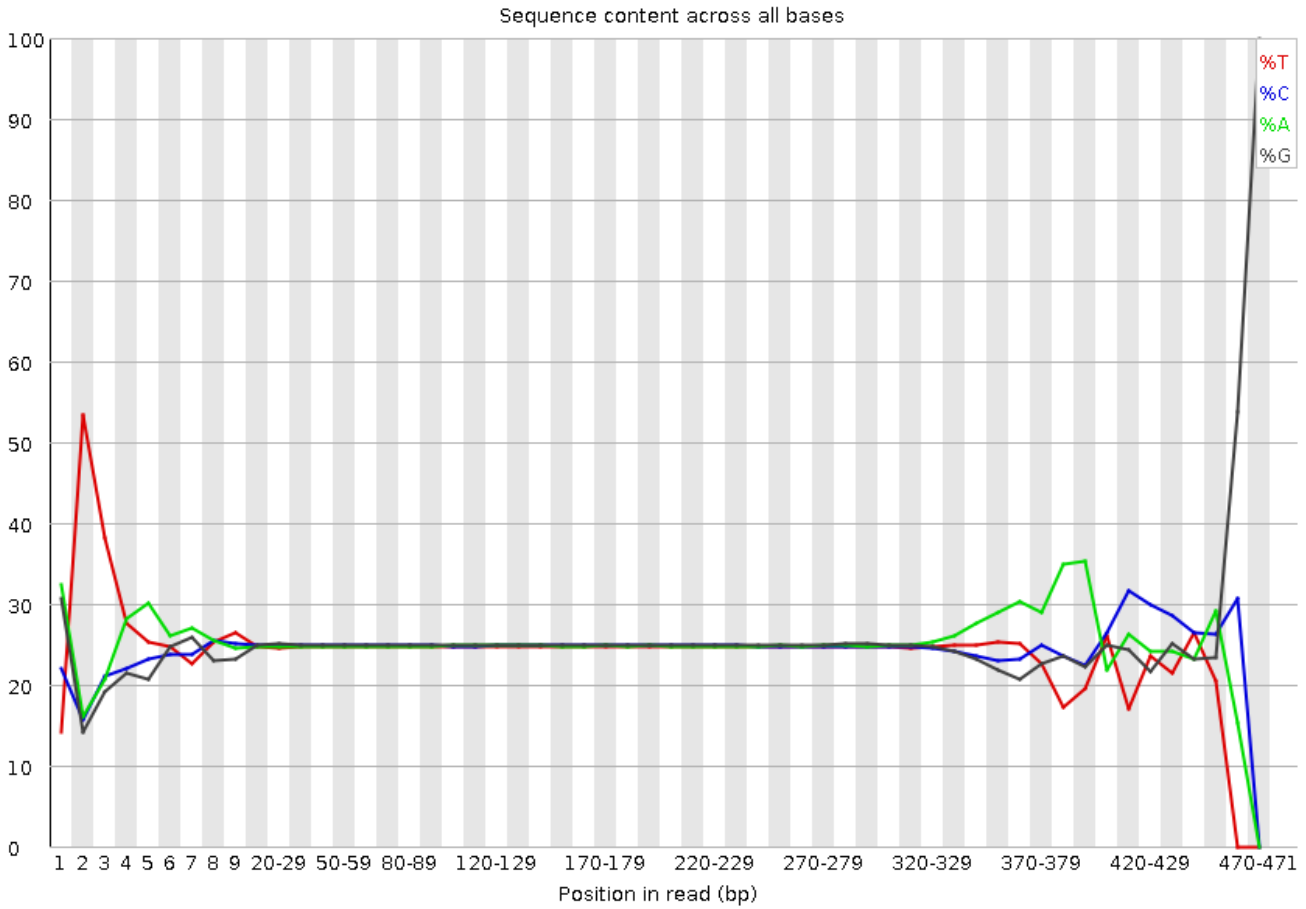
The blue line represents the mean quality

Minimum Phred quality score for right-side trimming: 25

Average Phred quality score for right-side trimming: 27

FastQC – quality check of raw data

Per base sequence content

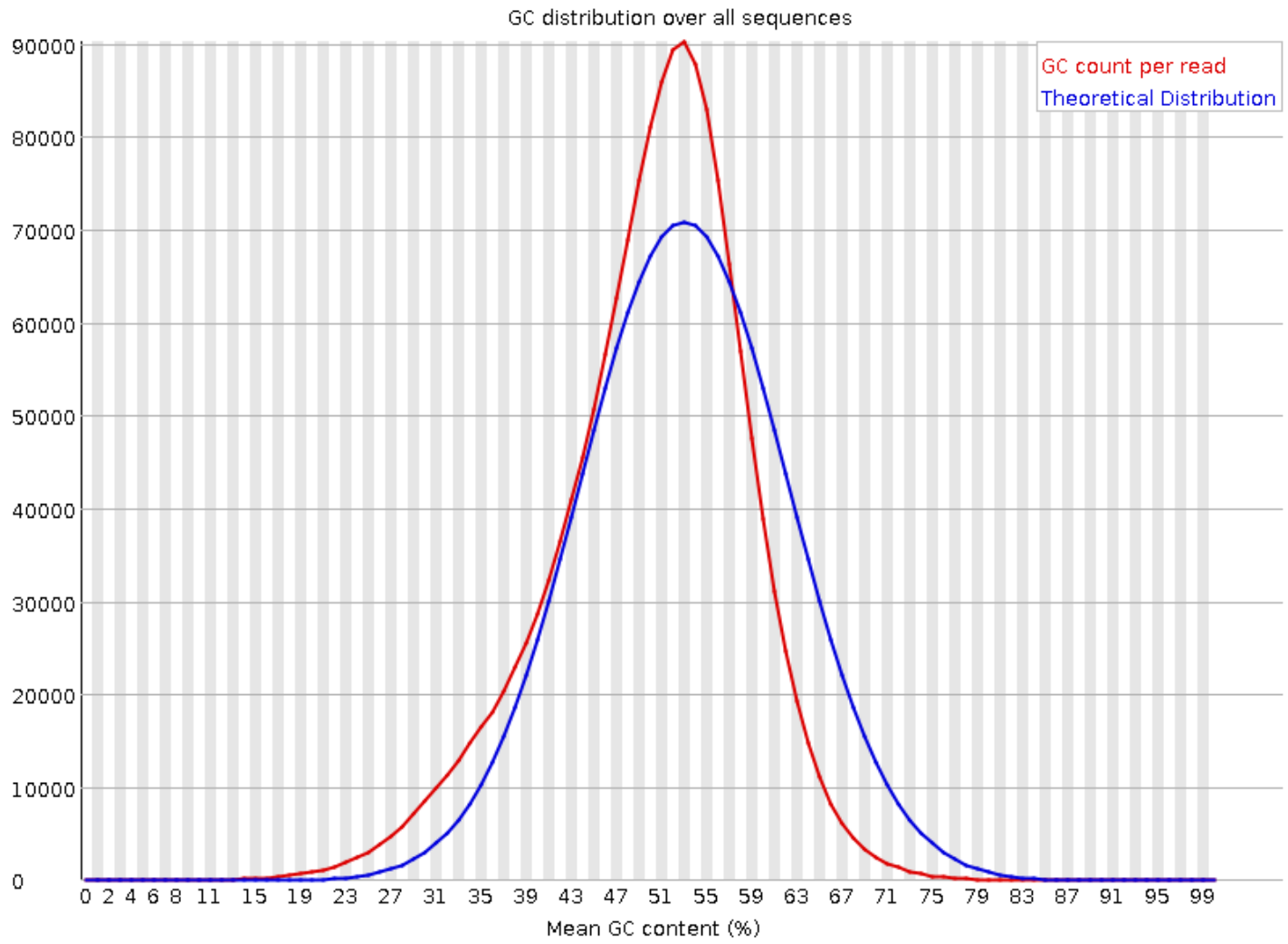


Left-side trimming: 10

Maximum length trimming: 330

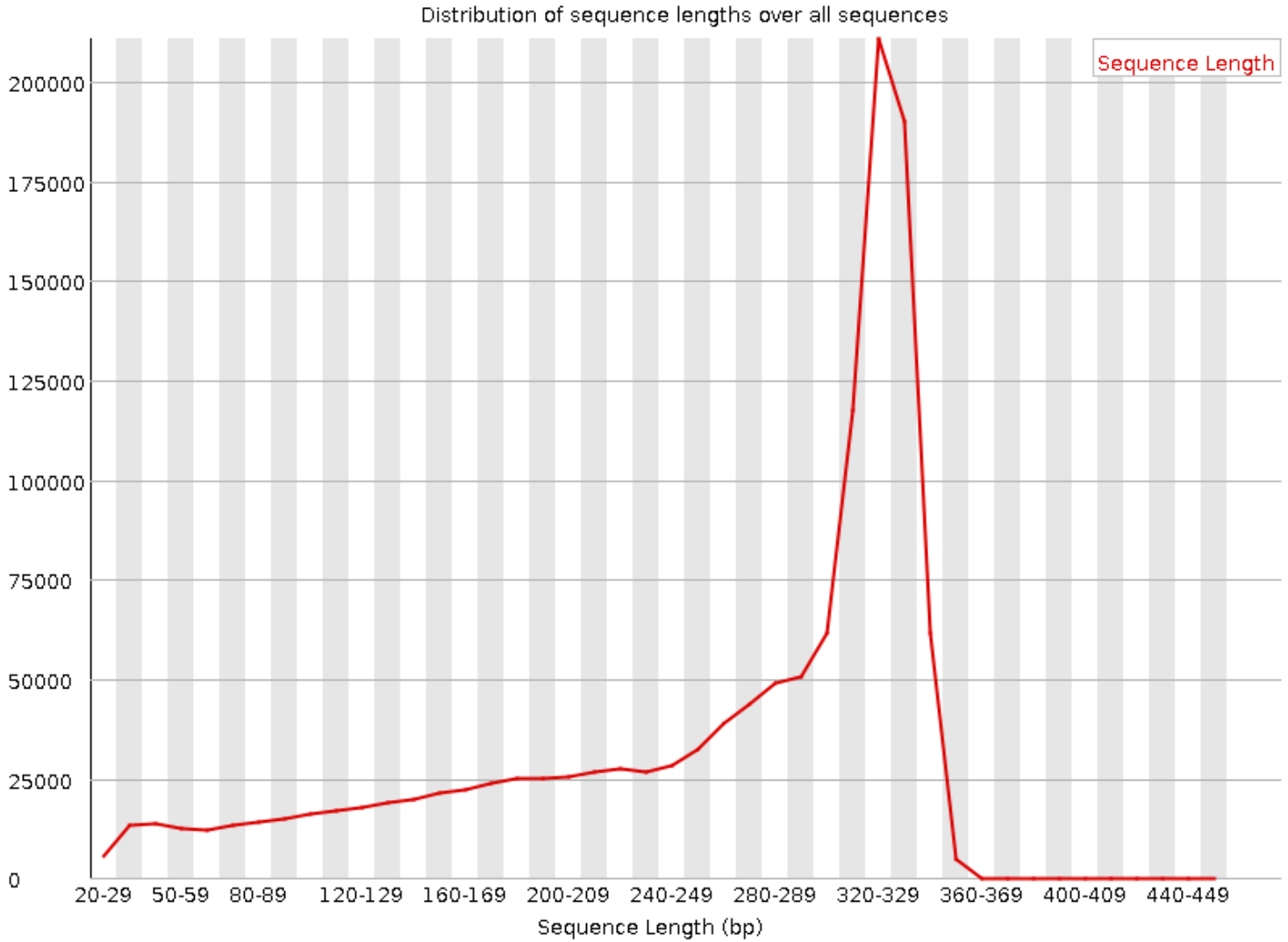
FastQC – quality check of raw data

Per sequence GC content



FastQC – quality check of raw data

Sequence Length Distribution



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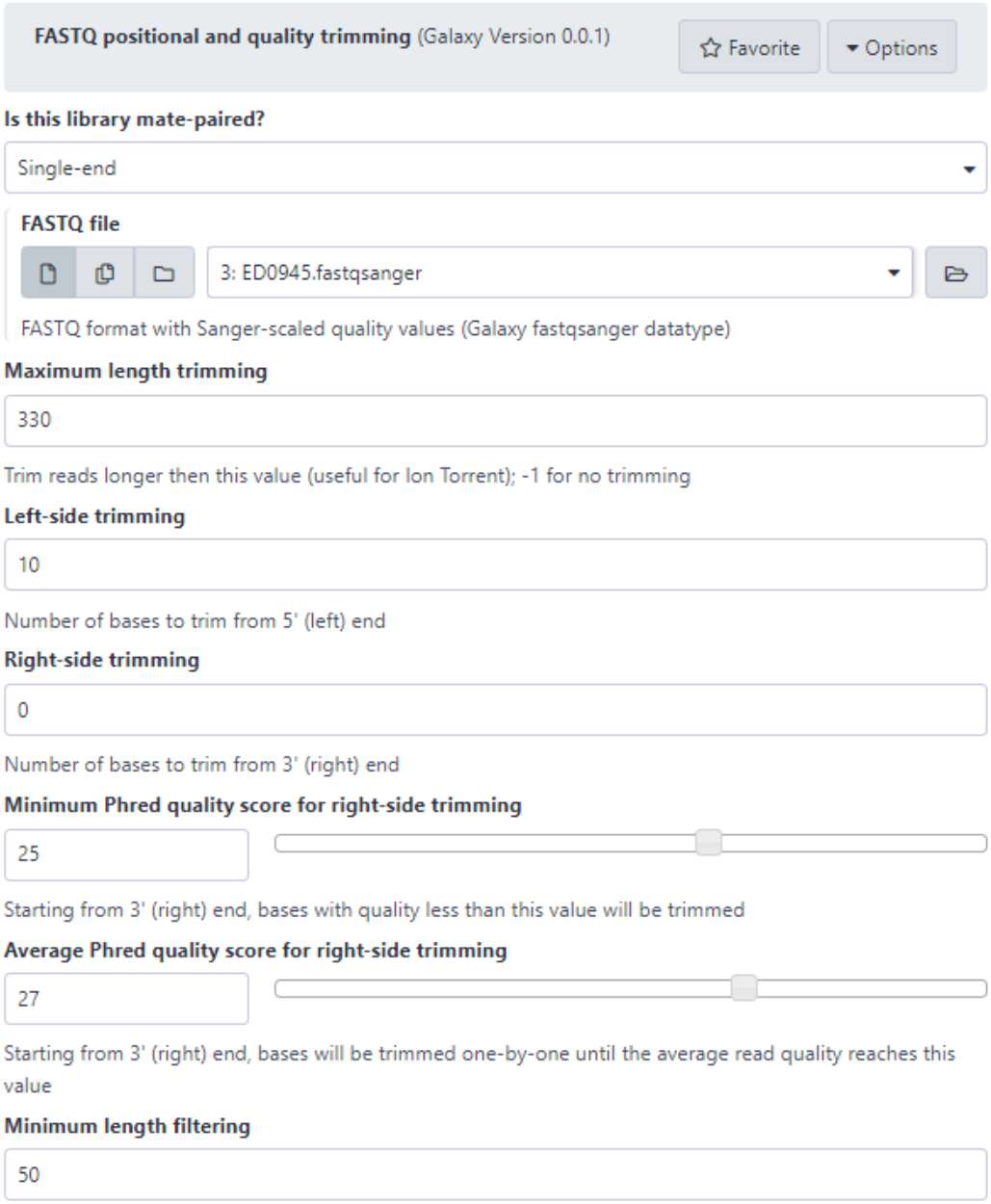
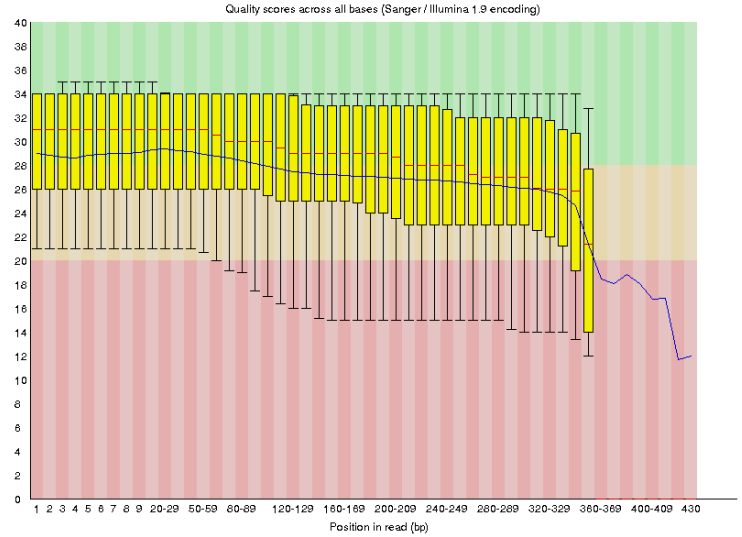
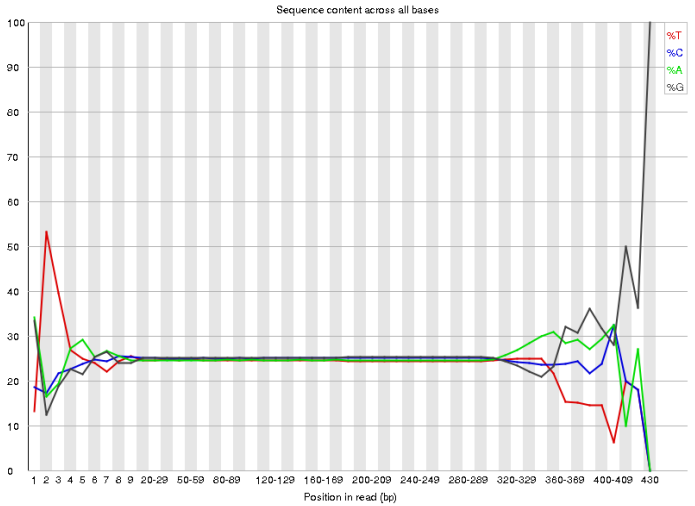


Before trimming





After trimming