

Session 2.2 – Quality assessment and read preprocessing

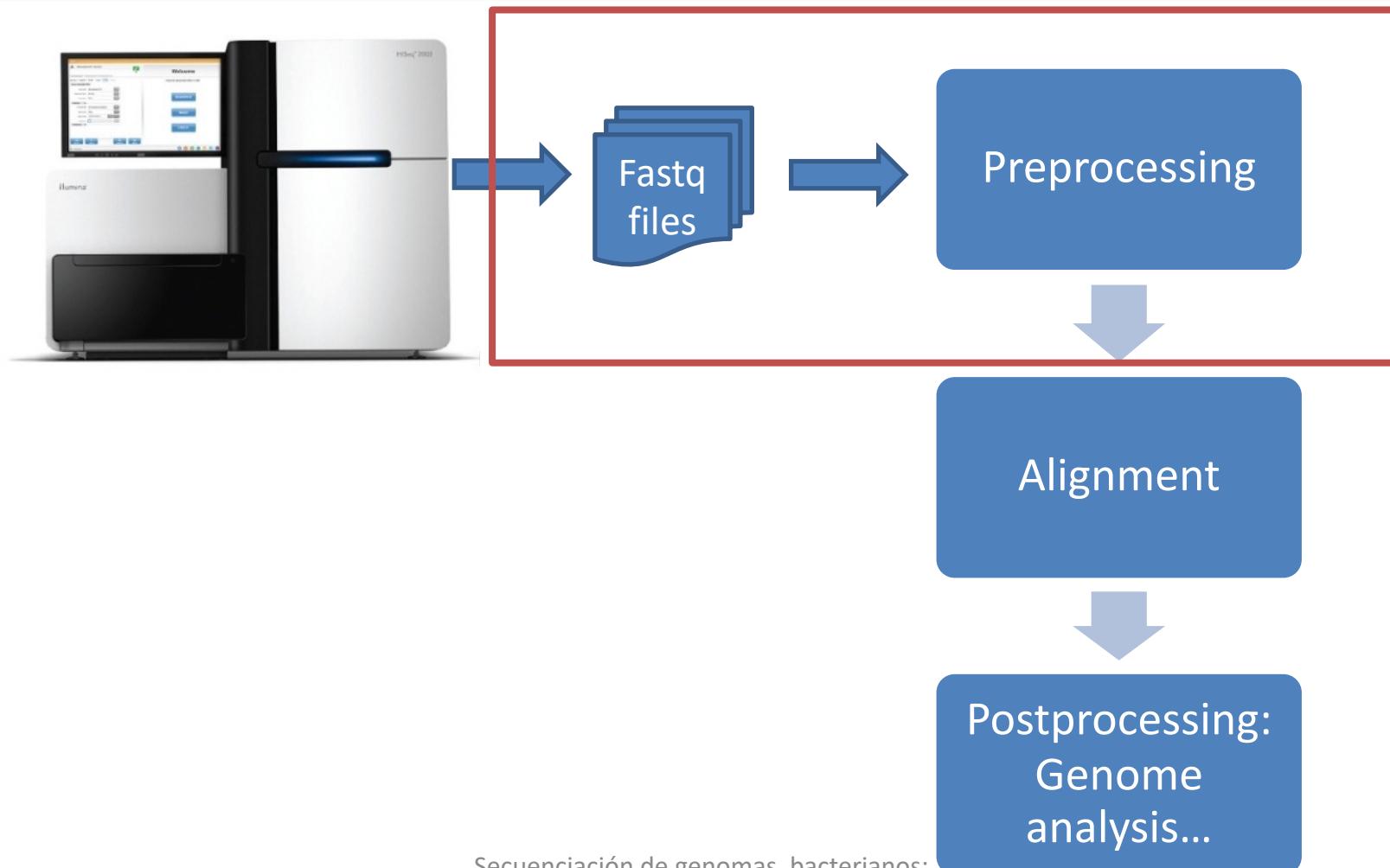
Sarai Varona

BU-ISCIII

Unidades Comunes Científico Técnicas - SGSAFI-ISCIII

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Programa Formación Continua, ISCIII

Step in the process



Raw output files format

Illumina



.fastq



454 .sff



SOLiD
.fasta
.qual



Nanopore
.fast5 or .fastq



PacBio RSII
Bax.h5
fasta

FASTQ format

- Is a FASTA file with quality information
- Within HTS, FASTA contain genomes y FASTQ reads

```
>SEQ_ID|  
AGCTTTCAATTCTGACTGCAACGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGC  
TTCTGAAGTGTTACCTGCCGTGAGTAAATTAAATTGACTTAGTCACAAATACTTAACCAA  
TATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACC  
ATTACCACCAACCATTACCAACAGGTAACGGTGCAGCTGACCGTACAGGAAACACAGAAAAAG
```

```
@SEQ_ID  
GATTTGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTGTTCAACTCACAGTTT  
+  
! ' * ( ( ( ***+ ) % % + + ) ( % % % ) . 1 *** - + * ' ) ) **55CCF>>>>CCCCCCCC65
```

Quality: must be 1 bit

FASTQ format

- Each base has an assigned quality score
 - Sequencing quality scores measure the probability that a base is called incorrectly
- How is it calculated?

Error probability

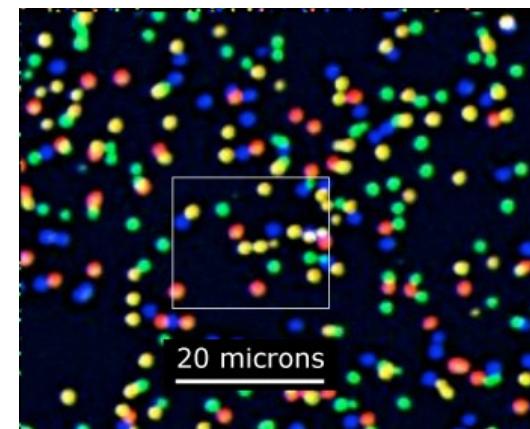
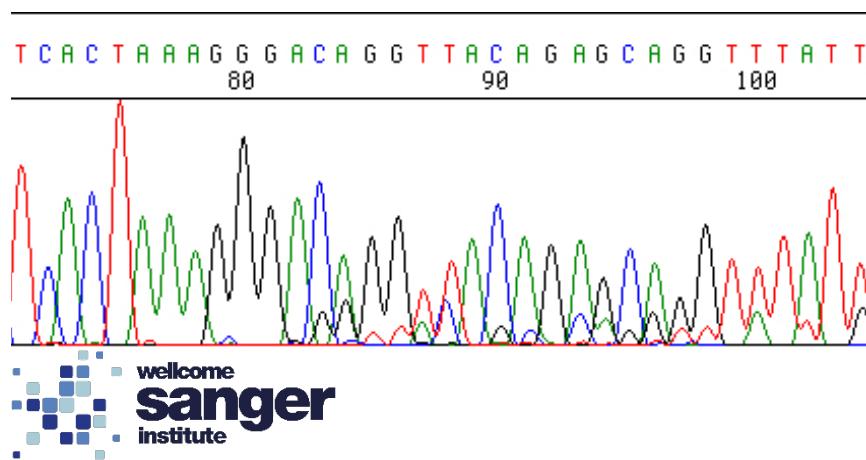
Phred
transforming

ASCII encoding

```
! ' ' * ( ( ( ***+ ) % % + + ) ( % % % ) . 1 *** - + * ' ' ) ) **55CCF>>>>>CCCCCCC65
```

Phred quality and error probability

- **Light intensity** is used to calculate the error probabilities
- Convert error probability into Phred score quality - Ewing B, Green P. (1998)
- Phred originated as an algorithmic approach that considered Sanger sequencing metrics, such as **peak resolution and shape**



Phred quality and error probability

- Convert error probability into Phred score quality - in real time on Illumina platforms
- Q scores are defined as a property that is logarithmically related to the base calling error probabilities (P)
- Phred quality range between 0-40 for Sanger and Illumina 1.8+
$$Q = -10 \log_{10} P$$

Phred Quality Score	Probability of Incorrect Base Call	Base Call Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

Phred quality and error probability

- Convert error probability into Phred score quality - in real time on Illumina platforms
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1.8+

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Phred Quality Score	Probability of Incorrect Base Call	Base Call Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

Error = 1 in 10.000

P = 0.0001

Q = $-10 \log_{10}(0.0001)$

Q = 40

Phred quality and error probability

- Convert Phred quality score into ASCII, a compact form, which uses only 1 byte per quality value

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

Q	P_error	ASCII									
0	1.00000	33 !	11	0.07943	44 ,	22	0.00631	55 7	33	0.00050	66 B
1	0.79433	34 "	12	0.06310	45 -	23	0.00501	56 8	34	0.00040	67 C
2	0.63096	35 #	13	0.05012	46 .	24	0.00398	57 9	35	0.00032	68 D
3	0.50119	36 \$	14	0.03981	47 /	25	0.00316	58 :	36	0.00025	69 E
4	0.39811	37 %	15	0.03162	48 0	26	0.00251	59 ;	37	0.00020	70 F
5	0.31623	38 &	16	0.02512	49 1	27	0.00200	60 <	38	0.00016	71 G
6	0.25119	39 '	17	0.01995	50 2	28	0.00158	61 =	39	0.00013	72 H
7	0.19953	40 (18	0.01585	51 3	29	0.00126	62 >	40	0.00010	73 I
8	0.15849	41)	19	0.01259	52 4	30	0.00100	63 ?	41	0.00008	74 J
9	0.12589	42 *	20	0.01000	53 5	31	0.00079	64 @	42	0.00006	75 K
10	0.10000	43 +	21	0.00794	54 6	32	0.00063	65 A			

- Phred+33 (Sanger and current Illumina). 0 Phred quality correspond to decimal 33, which is the symbol !

ASCII_BASE=64 Old Illumina

Q	P_error	ASCII									
0	1.00000	64 @	11	0.07943	75 K	22	0.00631	86 V	33	0.00050	97 a
1	0.79433	65 A	12	0.06310	76 L	23	0.00501	87 W	34	0.00040	98 b
2	0.63096	66 B	13	0.05012	77 M	24	0.00398	88 X	35	0.00032	99 c
3	0.50119	67 C	14	0.03981	78 N	25	0.00316	89 Y	36	0.00025	100 d
4	0.39811	68 D	15	0.03162	79 O	26	0.00251	90 Z	37	0.00020	101 e
5	0.31623	69 E	16	0.02512	80 P	27	0.00200	91 [38	0.00016	102 f
6	0.25119	70 F	17	0.01995	81 Q	28	0.00158	92 \	39	0.00013	103 g
7	0.19953	71 G	18	0.01585	82 R	29	0.00126	93]	40	0.00010	104 h
8	0.15849	72 H	19	0.01259	83 S	30	0.00100	94 ^	41	0.00008	105 i
9	0.12589	73 I	20	0.01000	84 T	31	0.00079	95 _	42	0.00006	106 j
10	0.10000	74 J	21	0.00794	85 U	32	0.00063	96 ^			

- Phred+64 (Solexa and Illumina 1.3-1.5)

Phred quality and error probability

- Phred 33 example

```
@HWI-ST731_6:1:1101:1322:1938#1@0/1
NTGACAAAGGGCTAATATCCAGAACATCTACA[AAGAACTTAAACAAATGT]ATAAGAATAAAAGTATAGTGCTAACAT
+
#1:BDDADFDFFD@F>BGFIIIB@CFHIHICAGBC9CBCBGGIGCFF??>GGHFHIGGEGI<FECGDE=FHCHEG=
```

$$P=0.0001 \rightarrow Q=-10 \log_{10}(0.0001) = 40 \rightarrow \text{ASCII} 33+40 = 73 \rightarrow !$$

$$P=0.001 \rightarrow Q=-10 \log_{10}(0.001) = 30 \rightarrow \text{ASCII} 33+30 = 63 \rightarrow ?$$

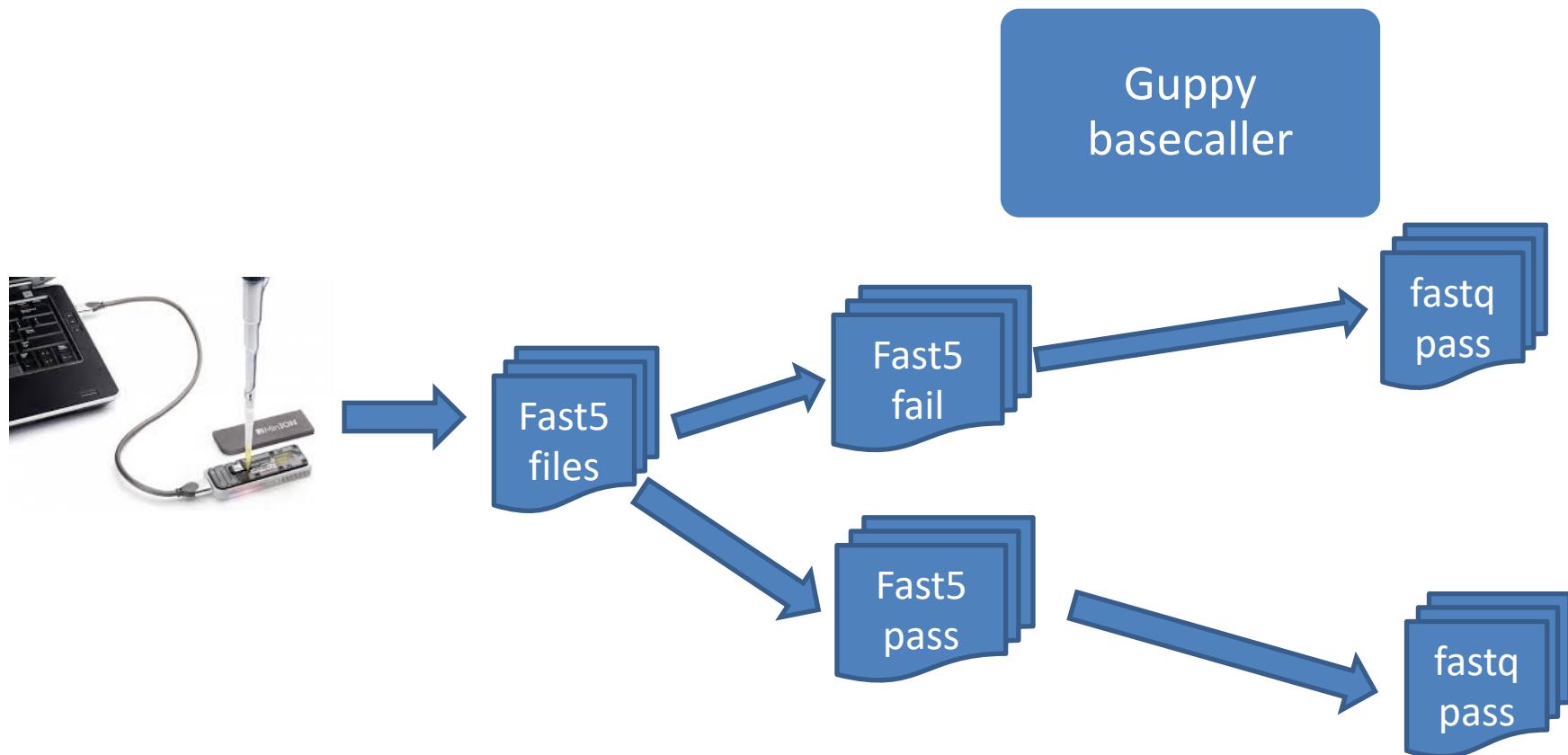
Quality encoding:	!"#\$%&'()*+,-./0123456789:;,<=>?@ABCDEFGHI
Quality score:	0.....10.....20.....30.....40

Error rate and Quality in Nanopore

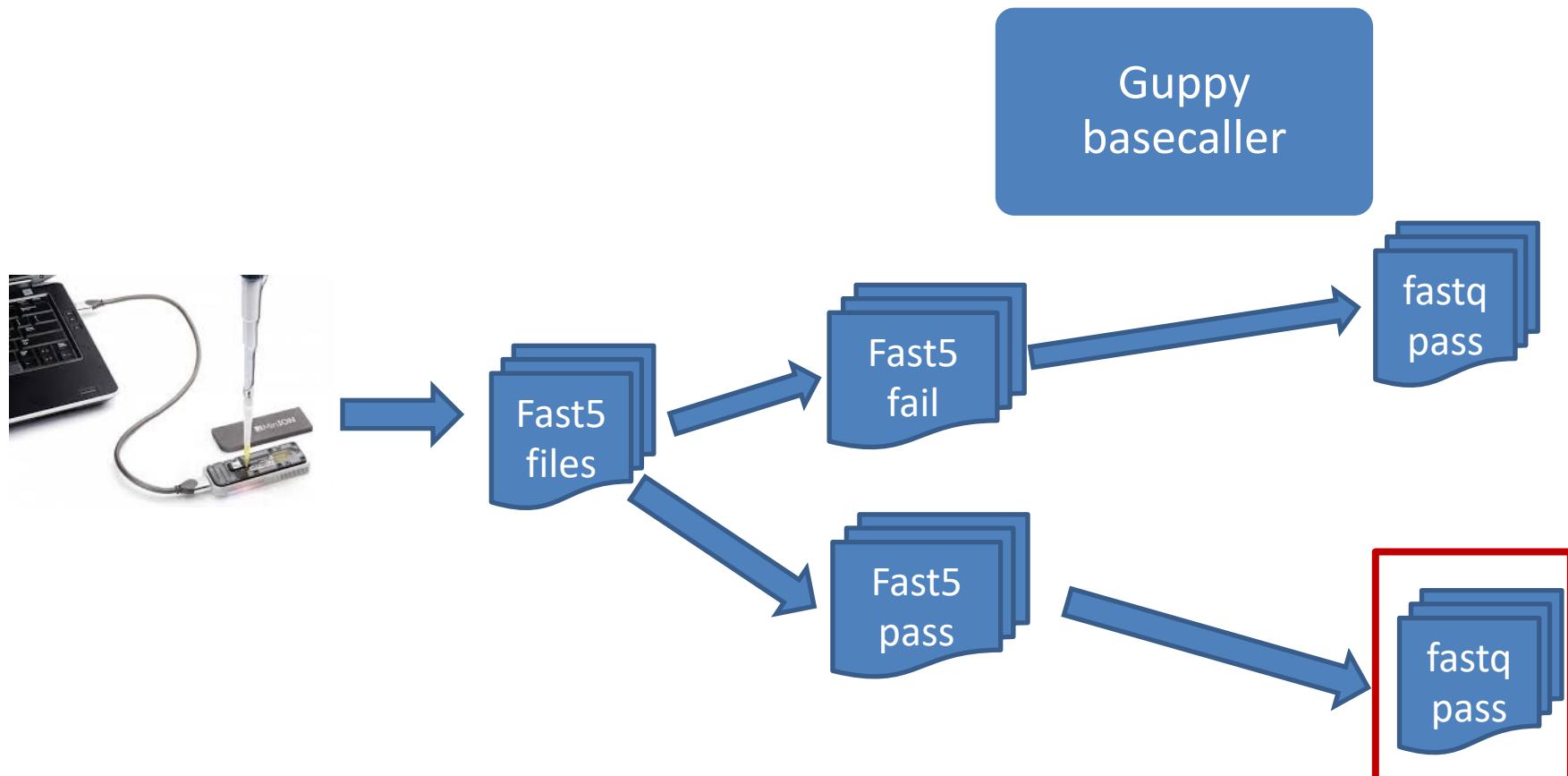


Clara Delahaye, Jacques Nicolas. Nanopore MinION long read sequencer: an overview of its error landscape. 2020. fffhal-03123133f

Error rate and Quality in Nanopore

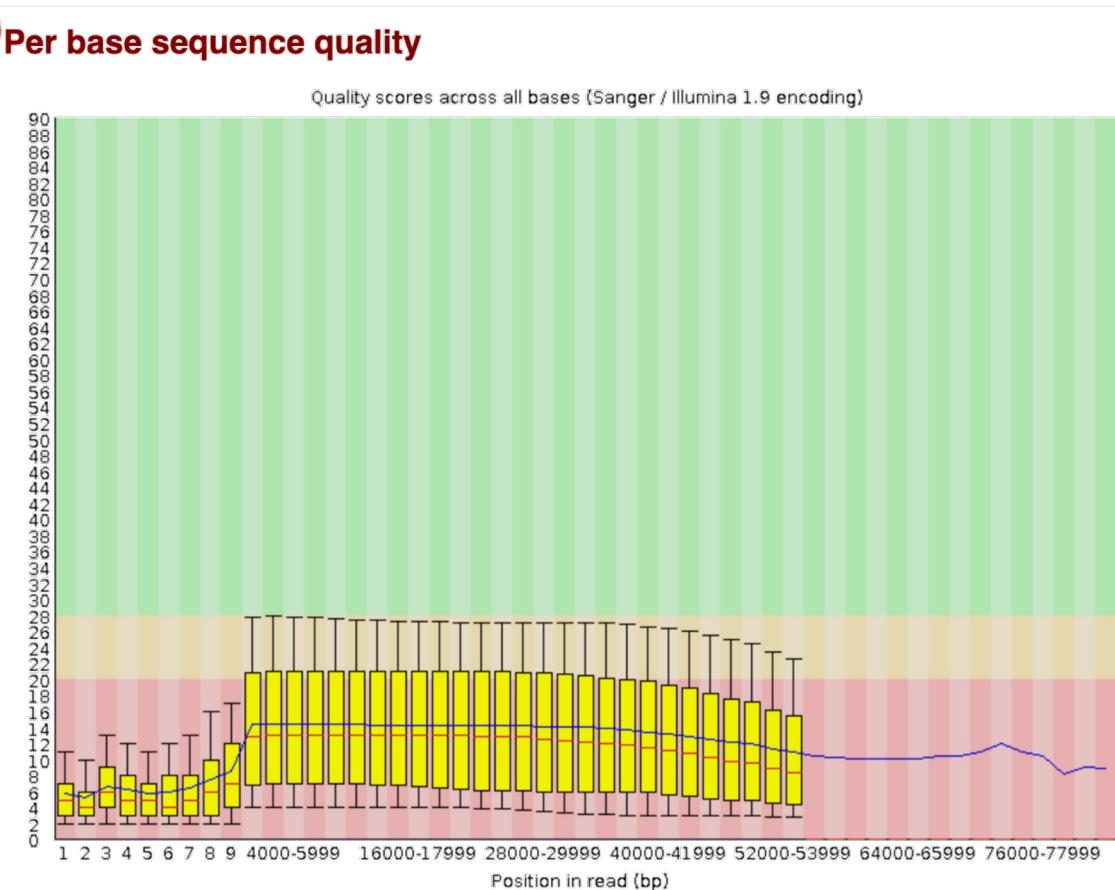


Error rate and Quality in Nanopore



Error rate and Quality in Nanopore

- Nanopore quality score (Q) does not follow Phred scores
- To estimate error rate (E) (locally and at read level):
$$E = 0.015Q^2 - 1.15Q + 24$$

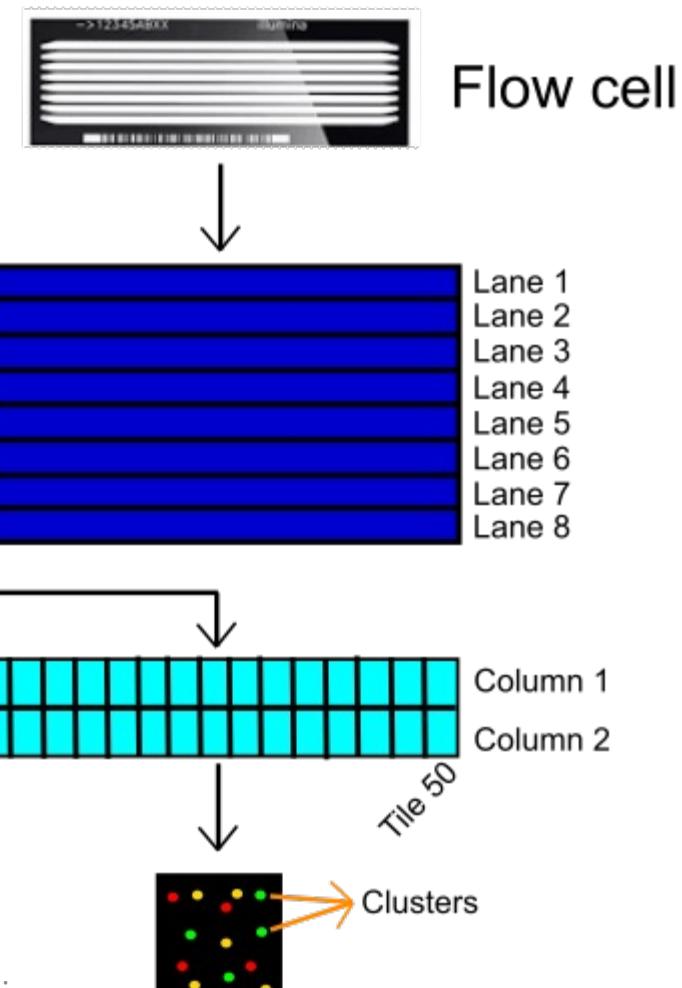


FASTQ format

Illumina read header

Sequence Header												+Sequence ID
a	b	c	d	e	f	g	h	i	j	k		
@HWI-ST486	:166:	C06K9ACXX	:	7	:	1101	:	1443	:	1995	1:N:0:	ACAGTG

- a. unique instrument name
- b. run id
- c. flowcell id
- d. flowcell lane
- e. tile number within the flowcell lane
- f. x-coordinate of the cluster within the tile
- g. y-coordinate of the cluster within the tile
- h. the member of a pair, 1 or 2 (paired-end or mate-pair reads only)**
- i. Y if the read fails filter (read is bad), N otherwise
- j. 0 when no control bits are on
- k. index sequence



FASTQ format

Illumina read header

Sequence Header	+Sequence ID
a b c d e f g h i j k	
@HWI-ST486:166:C06K9ACXX:7:1101:1443:1995 1:N:0:ACAGTG	

a. unique instrument name

- b. run id
- c. flowcell id
- d. flowcell lane
- e. tile number within the flowcell lane
- f. x-coordinate of the cluster within the tile
- g. y-coordinate of the cluster within the tile

h. the member of a pair, 1 or 2 (paired-end or mate-pair reads only)

- i. Y if the read fails filter (read is bad), N otherwise
- j. 0 when no control bits are on
- k. index sequence

```

@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:2458:1027 1:N:0:ACAGTG
AGAAAAAAACCTTGGANGGAAAAAAATCAGACATTCTAGAGGTGGAAGGCAAACCTGAACAAAGAAATAATTCA
+
DGGGEDHHHHGGGFEBACBCA<?HHHHBHHHHHHHHDDHHHEHEFEGGGGG/GGDDDGHFHGFCHFHHHEH8
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3082:1029 1:N:0:ACAGTG
GGTAATACAGACTGANATGATCAAAGGCATGCTGGAAACAAACCTATTAAAGATAAGCTGGATCAAGCTTCATT
+
B:B:B?BB:-=55177#55877<775EDD>E=B?BBBBGGGDDA@G>GGGGGG@)EEEEBEG>GGGGGGGAAA?<D
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3185:1033 1:N:0:ACAGTG
TCTGGGACATTGCTCNTGGCTGGAGTCACCTGCTGGACATTGCTCAGGGCTGGAGACACGTGTTGGAGGGAC
+
BC??A66;)74781<#??;452.27'64(8,851DDG8GB######
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3268:1033 1:N:0:ACAGTG
ATTCAAATTAGAAGANAGTTGATGTTCTCATGATGCCAAAATTCACTGAGAAAACCTTTTTAAGCCAC
+
IIIIIIIIIFFFF#ABACFEFFIFIIFIHE@IIIIIIIIHHIIIFIH>HHIHIFGDIIIIIIGFHIEGH
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3400:1035 1:N:0:ACAGTG
TCCTGCTTAGGAGANTCCTCATGCTCTGACAGGATGCTCTATGTGAGTTGAGCTGGCTTCTCACTTTATAG
+
IIIIIIIIHHIIGGEGG#AAC@?=BHIIIIIIHHIIHIIHIIHGHGIGIHHGIGIHHGEGGGGGH@EFGCEFAB
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3962:1033 1:N:0:ACAGTG
CCACCAACACAGTCTNCACCTCTGGTGTGGATAGATTTGCACCTTCCATCCTCAGGTTCAAATAGC
+
HHFHHDDHH>C?CA#EEEE>?A?>HHDGHEGBGBCEEHHF8HEEEHECH,=>==EAEE>BEBBAEAACAB
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:4491:1028 1:N:0:ACAGTG
AGAGAGAGAGAGAGAGAGAGACTCTGGAGATGCCAAGCACAAGCCTGCAAGAGTCCCAGCAAAGAAAATAAAAAA
+
GADGEGGEGGBBB?B#Q=@@72;64GGGFGB>GGGBDG<DBGB<DA??/?#####

```

ASCII-coded (0-40):

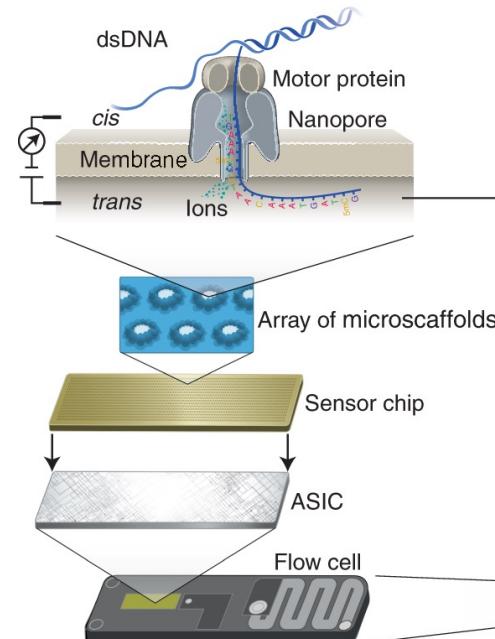
- “!”#\$%” lowest quality
- “FGHI” highest quality

FASTQ format

Nanopore read header

```
@d76be4fb-11a9-47e7-90be-c4f15591e0d9
runid=ba02134f00f2059e7b2dc248113c02f76577b101 read=11 ch=142 start_time=2019-06-
27T11:09:03Z flow_cell_id=FAH59799 protocol_group_id=k6963 sample_id=k6963
```

1. @read identifier
2. run-id
3. read-id
4. channel
5. start_time
6. flow_cell_id
7. protocol_group_id
8. sample_id



FASTQ format

Nanopore read header

@d76be4fb-11a9-47e7-90be-c4f15591e0d9
runid=ba02134f00f2059e7b2dc248113c02f76577b101 read=11 ch=142 start_time=2019-06-27T11:09:03Z flow_cell_id=FAH59799 protocol_group_id=k6963 sample_id=k6963

1. @read identifier
 2. run-id
 3. read-id
 4. channel
 5. start_time
 6. flow_cell_id
 7. protocol_group_id
 8. sample_id

@d76be4fb-11a9-47e7-90be-c4f15591e0d9 runid=ba02134f00f2059e7b2dc248113c02f76577
b101 read=11 ch=142 start_time=2019-06-27T11:09:03Z flow_cell_id=FAH59799 protocol_group_id=k6963 sample_id=k6963
CATTGTACTGATTCAACATATTGCTGTTTCATCAAGGGAGAAAGTAATGACAGCGCATCGCAGTGAAAAGAGACT
CGATGGGCGCCGTCAAGGGGTCCCAGCCGATAAAACTCTGGGGCGCCCAGACCAGCGCTCGCTGGAAACATTCCGCATC
TCGACCGAAAAAATGCAGAGGCCAATGCCACGCCAGCATGACCAGGCAGCAGCGAAAGGGTGTGAGATCTGGACTGTG
ACAACGGAAAAAAGCCAGGGCAGTCGCGCCATGACGAAGTGCTGGCCGAAAGCACCGCAGAGGTTCCAGCTATC
TGGCAGACCGGCTCCAGCAAAGCAATATGAGAATATGAACGAGGTGCTGGCCAGCCGCTCAATGAACGTCTGGCGGA
GAACGGGGATAGCGCGAAAATTATAATGACGATGTAGATAAACAGAAGCTCAAATGATGTATTCCC Captura de pantalla 2021-11-18 a las 13.01.51 Imagen PNG
+ Captura de pantalla 2021-11-18 a las 13.01.51 Imagen PNG
##\$& "#\$\$&\$17)\$##%#%"'**)*%"'3679***((70>->>B>;>`&,400+&'89?344.&'0=N61%+\$33
)1>;7/++))&##%%(38?;=@?8-?A>4432&(,35+;6%\$##+'321%+\$%)*+\$%')\$((158; ;%2/10..
8+>66A:9?>79-+-*\$%+, .-/-*\$**, 1680('(+-('**,%%.)))600/(+.*))\$#&'#2222,<==B:9,6-
-%%\$.///*1B9<;)@&20. --53729</99246##+5))/->;7(*41##+6&33*'(%*13-\$8'9;8/'++*)46
8/)+,+56%;2207#\$(0.7;6:A2--('+-,.%"%&%', =[A74973](#)/.%&'()\$+\$)*,;5'%#"')5\$()*)+)
%*610&3>2++%((0366*&#&)8:=@2-20%&"\$")\$-,1)=8/+&&9/D3C>446%&(*+1,
@6d14c02c-1950-46f3-804c-3391a8020324 runid=ba02134f00f2059e7b2dc248113c02f76577
b101 read=6 ch=451 start_time=2019-06-27T11:09:04Z flow_cell_id=FAH59799 protocol_group_id=k6963 sample_id=k6963 Captura de pantalla 2021-11-18 a las 9.56.12 Imagen PNG
GGTATTACTCGTTCAGTTACGTGTCTCGCTTCGGTTGATGCCGTAAACAGCAGCGGCCACCCGAGGTGATATCT
CCCTGCGCAGCGCATTGCCAGAACCAACCGGCGCAGCAGTAGTCTGCATGAATAACCGAGCAGGCCAGTAGAAATCGGG
GCAATAAACAGGTACTTAATTCACTTTGTACATCTCATGCAAAAAAAACTTAAAGCTCCGAAACAGGGACTTATAA Captura de pantalla 2021-11-18 a las 9.56.14 Imagen PNG
run_id report run_id report run_id report run_id report

Sequencing quality assessment

- To asses quality, software uses **Phred per-base quality score** is used
- Is the **first quality control step** after sequencing. There should be one after every step of the analysis
- After quality assessment user can know how **reliable** are their datasets
- QC will determine the next **filtering** step
- Filtering decisions will **impact** directly in **further analysis**
- Many other steps also use this quality as variable in their **algorithms**

Sequencing quality assessment: Artifacts

HTS methods are bounded by their technical and theoretical limitations and sequencing errors cannot be completely eliminated (Hadigol M, Khiabanian H. 2018)

- **Artifacts in library preparation**

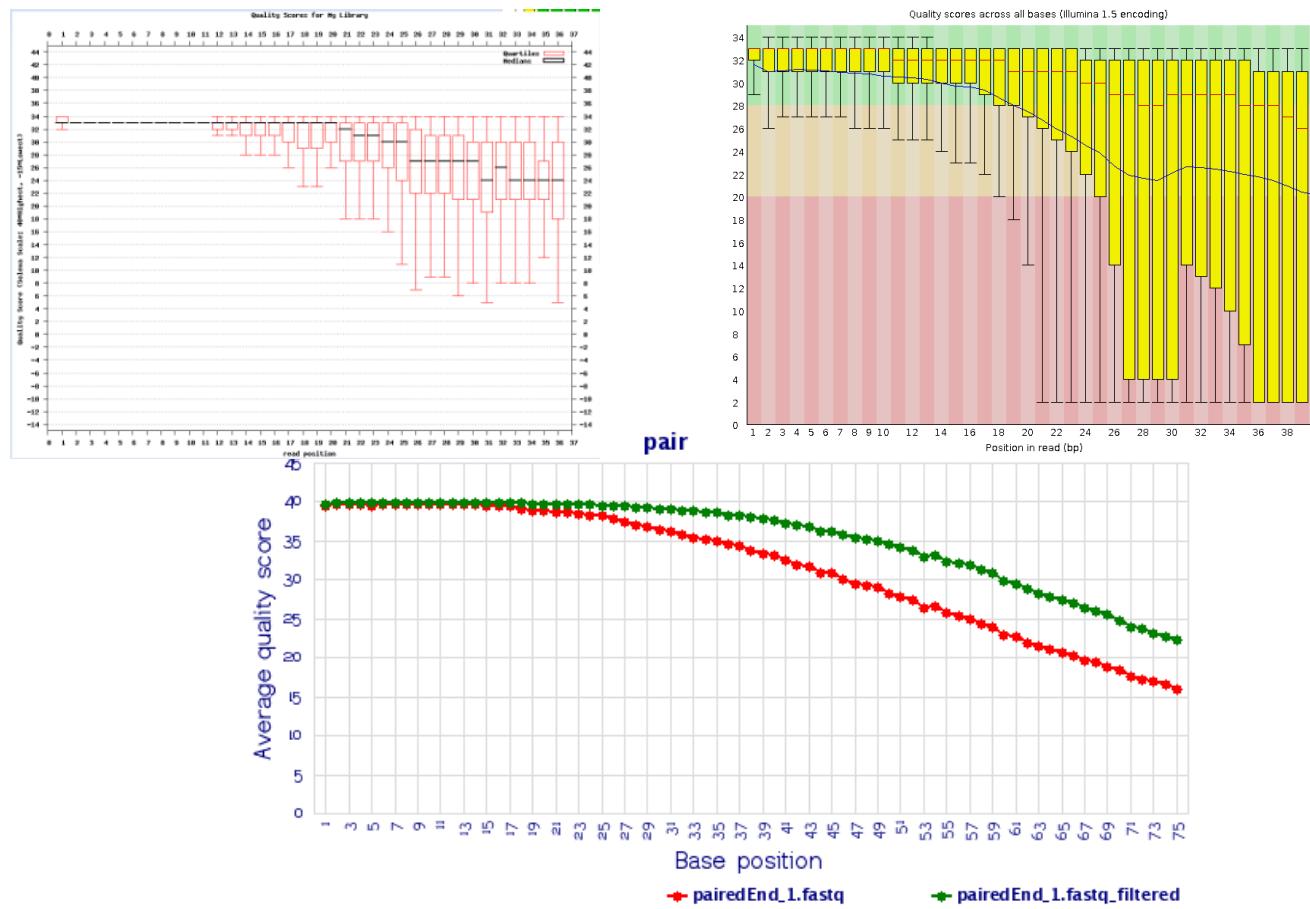
- Remaining adapters
- High rate of duplicates
- GC regions bias
- Polymerase error rate
- DNA damage during breakdown

- **Artifacts during sequencing**

- Low quality in sequence ends (Phasing: cluster loose sync)
- Complication in certain regions:
 - Repetitions
 - Homopolymers
 - High CG content

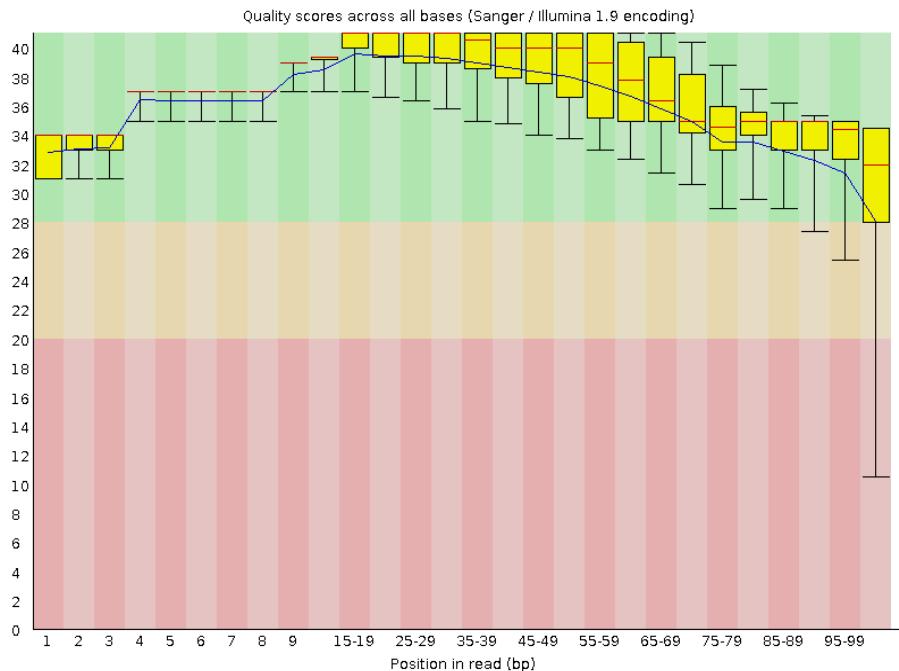
Sequencing quality assessment

- FastQC, fastx-toolkit, sfftools, NGSQCToolkit, etc...

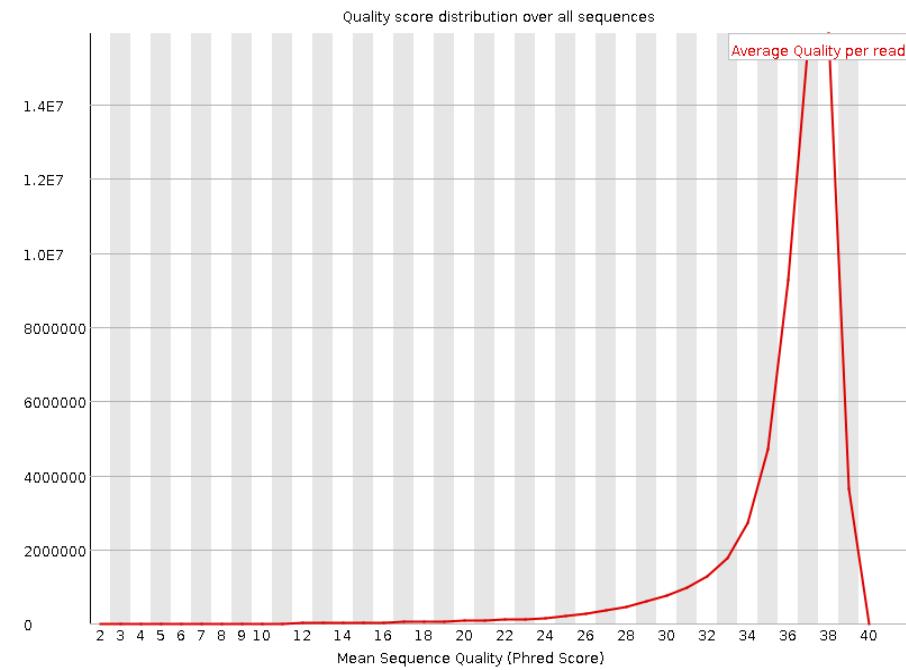


Sequencing quality assessment: FastQC

Per base sequence quality



Per sequence quality scores



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

Sequencing quality assessment: fastp

- **Fastp**
fastp report

Summary	
General	
fastp version:	0.20.1 (https://github.com/OpenGene/fastp)
sequencing:	paired end (149 cycles + 149 cycles)
mean length before filtering:	116bp, 116bp
mean length after filtering:	117bp, 117bp
duplication rate:	1.704150%
Insert size peak:	95
Detected read1 adapter:	CACCTAAGTTGGCGTATACGCGTAATATCTGGGTTCTACAAAATCATACCGTCCT
Detected read2 adapter:	CACCTAAGTTGGCGTATACGCGTAATATCTGGGTTCTACAAAATCATACCGTCCT
Before filtering	
total reads:	1.296756 M
total bases:	151.424921 M
Q20 bases:	143.112834 M (94.510754%)
Q30 bases:	137.905419 M (91.071812%)
GC content:	40.410939%
After filtering	
total reads:	854.250000 K
total bases:	100.537720 M
Q20 bases:	99.598139 M (99.065444%)
Q30 bases:	97.968091 M (97.444115%)
GC content:	39.665634%
Filtering result	
reads passed filters:	854.250000 K (65.875924%)
reads with low quality:	352.272000 K (27.165635%)
reads with too many N:	84 (0.006478%)
reads too short:	90.150000 K (6.951963%)



FastQC: Basic Statistics

- Self defined overall stats
 - Encoding: Phred33 or Phred64



Basic Statistics

Measure	Value
Filename	bad_sequence.txt
File type	Conventional base calls
Encoding	Illumina 1.5
Total Sequences	395288
Sequences flagged as poor quality	0
Sequence length	40
%GC	47



Basic Statistics

Measure	Value
Filename	good_sequence_short.txt
File type	Conventional base calls
Encoding	Illumina 1.5
Total Sequences	250000
Sequences flagged as poor quality	0
Sequence length	40
%GC	45

FASTQ format

Illumina read header

Sequence Header

+Sequence ID

a b c d e f g h i j k

@HWI-ST486:166:C06K9ACXX:7:1101:1443:1995 1:N:0:ACAGTG

a. unique instrument name

- b. run id
- c. flowcell id
- d. flowcell lane
- e. tile number within the flowcell lane
- f. x-coordinate of the cluster within the tile
- g. y-coordinate of the cluster within the tile

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```

@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:2458:1027 1:N:0:ACAGTG
AGAAAAAAACCTTGGANGGAAAAAAATCAGACATTCTAGAGGTGGAAAGCAAAACTGAACAAAGAAATAATTACA
+
DGGGEDHHHHGGGFEBACBCA<?HHHHBHHHHHHHHDDHHHEHEFEGGGGGGG/GGDDDGHFHGFCHFHHHEH8
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3082:1029 1:N:0:ACAGTG
GGTAATACTACAGACTGANATGATCAAAGGCATGCTGGAAACAAACCTATTAGATAAGCTGGATCAAGCTTCATT
+
B:B:B?BB:-=55177#55877<775EDD>E=B?BBBBGGGDDA@G>GG(GGG@)EEEEBEG>GGGGGGAAA?<D
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3185:1033 1:N:0:ACAGTG
TCTGGGACATTGCTCNTGGCTGGAGTCACCTGCTGGACATTGCTCAGGCTGGAGACACGTGTTGGAGGGAC
+
BC??A66;)74781<#??;452.27'64(8,851DDG8GB?#####/######
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3268:1033 1:N:0:ACAGTG
ATTCAAATTAGAAGANAGTTGATGTTCTCATGATGCCAAATTTCACTGAGAAAACCTTTTTAAGCCAC
+
IIIIIIIIIFFFF#ABACFEFFIFIIFIHE@BBBBBBBBBHIIFIIF>HHIHFIDIIIIIGFHIEGH
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3400:1035 1:N:0:ACAGTG
TCCTGCTTAGGAGANTCCTCATGCTCTGACAGGATGCTCTATGTAATTGAGCTGGCTTCTCACTTTATAG
+
IIIIIIHIIHIGGEGG#AAC@?=BHIIIIIIHHIIIIHIIHGHIIHGIGIGHGEGGGGHG@EFGCEFAB
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:3962:1033 1:N:0:ACAGTG
CCACCAACACAGTCTNCACCTCTGGTGTGGATAGATTTGACCTTCCATCCTCCAGGTTCAAATAGC
+
HHFHHDDHH>C?CA#EEEE>?A?>HHDGHEGBGBCEEHHF8HEHEHECH,=>==EAEE>BEBBAEAACAB
@HWUSI-EAS1752R:21:FC64JUKAAXX:3:1:4491:1028 1:N:0:ACAGTG
AGAGAGAGAGAGAGAGAGAGACTCTGGAGATGCCAAGCACAAGCTGAAAGAGTCCCAGCAAAGAAAATAAAAAA
+
GADGEGGEGGBBB?B#Q=@@72;64GGGFGB>GGGBDG<DBGB<DA??/?#####

```

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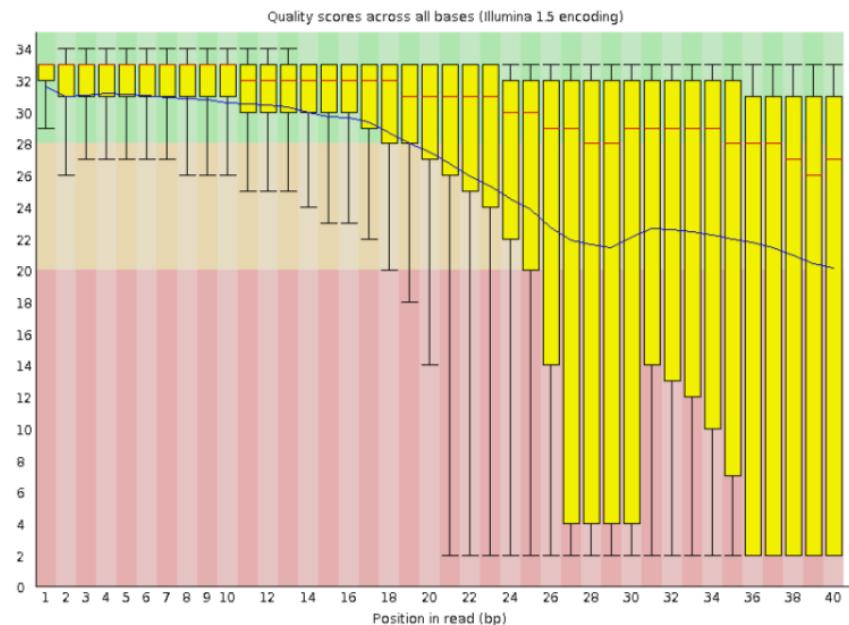
Basic Statistics

Measure	Value
Filename	good_sequence_short.txt
File type	Conventional base calls
Encoding	Illumina 1.5
Total Sequences	250000
Sequences flagged as poor quality	0
Sequence length	40
%GC	45

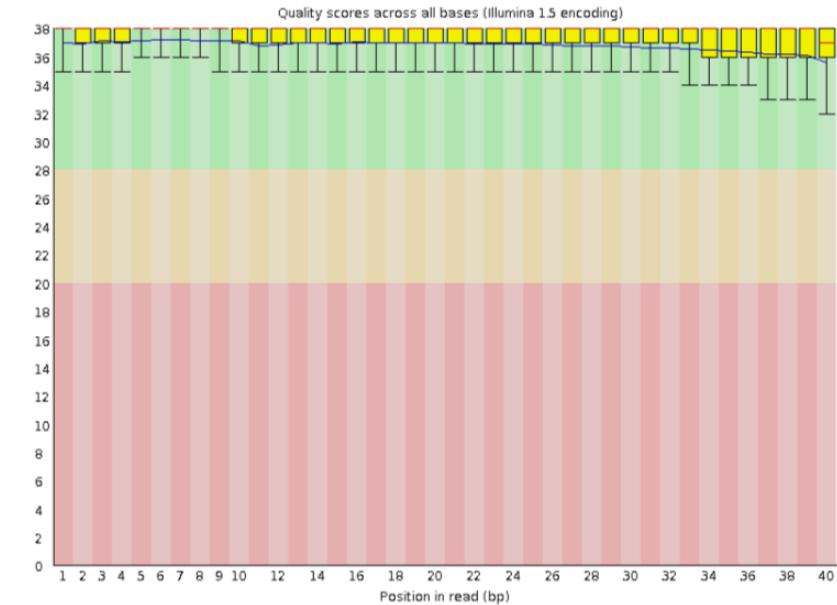
FastQC: Per base sequence quality

- Overview of the range of quality values across all bases at each position in the FastQ file
- Median, inter-quartile range (25-75%), 10-90% points, mean quality

✖ Per base sequence quality



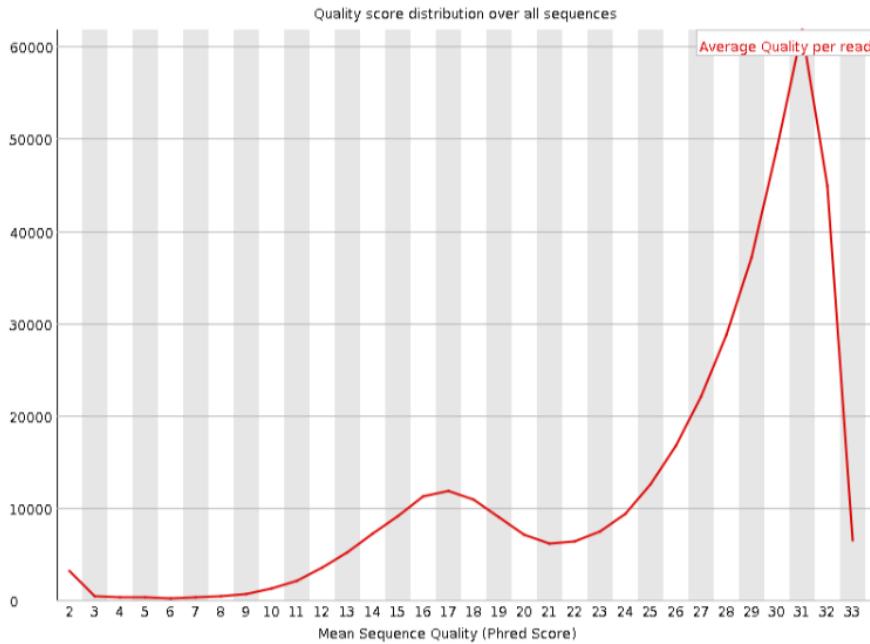
✓ Per base sequence quality



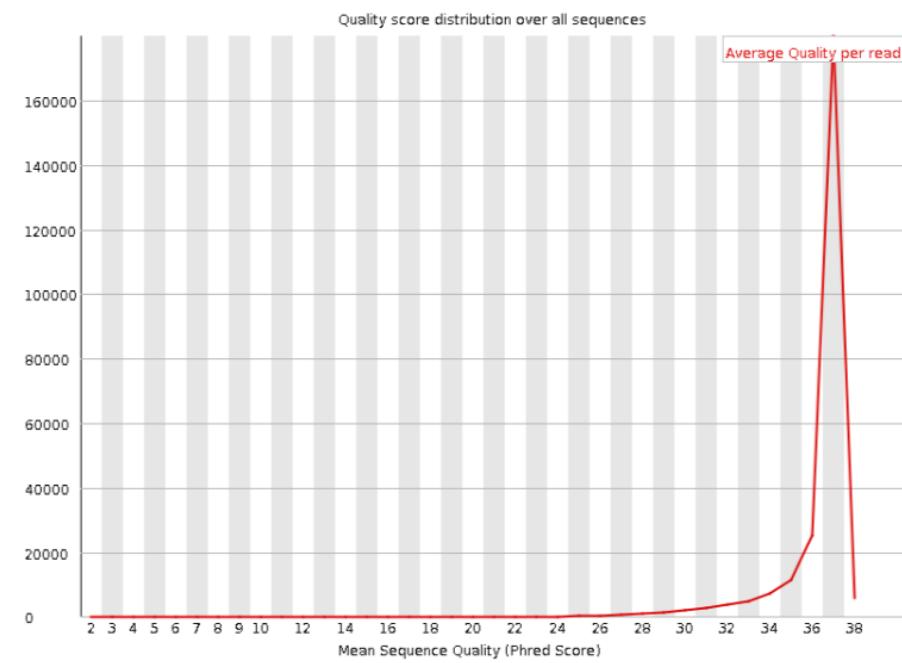
FastQC: Per sequence quality score

- Number of sequences with the same mean quality

Per sequence quality scores



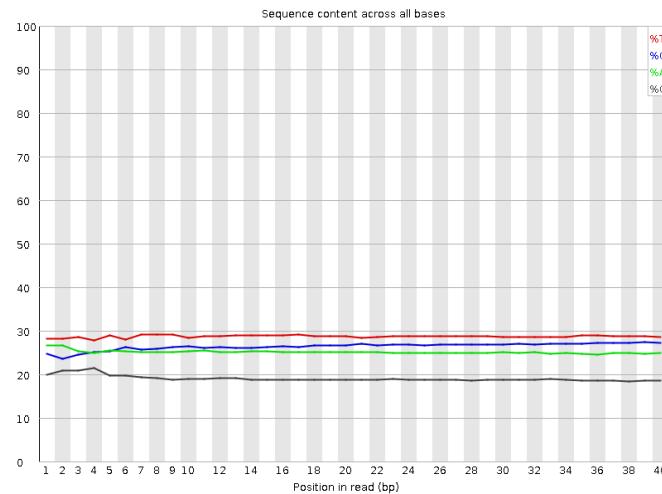
Per sequence quality scores



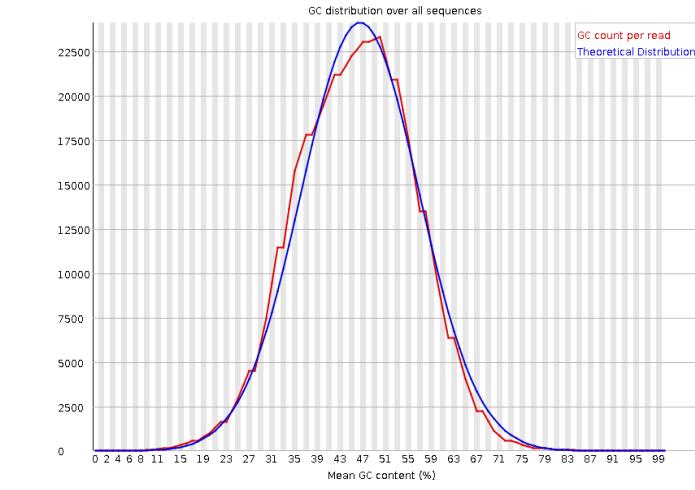
FastQC: Nucleotide related errors

- How expected nucleotide distribution deviates from expected
 - Per base sequence content
 - Per base GC content
 - Per sequence GC content
 - Per base N content

⚠ Per base sequence content

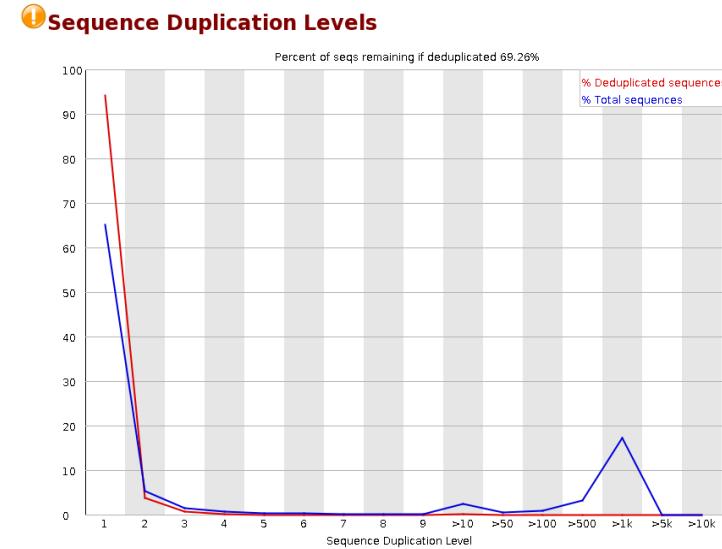
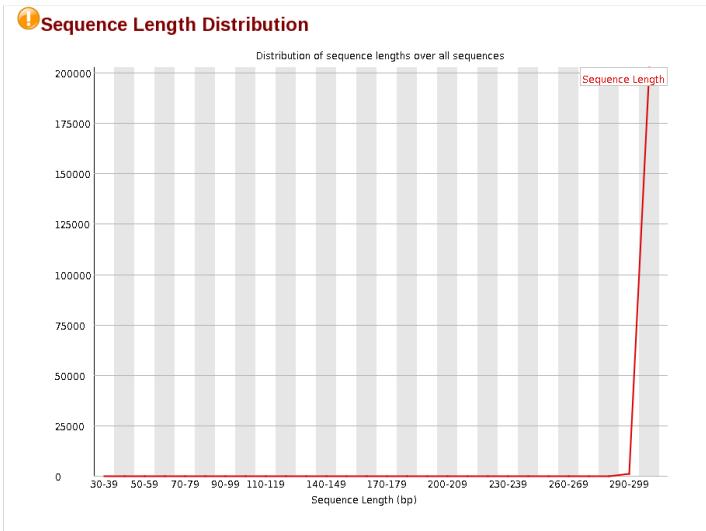


✅ Per sequence GC content



FastQC: Sequence related errors

- How expected nucleotide distribution deviates from expected
 - Sequence Length Distribution - Fragments
 - Sequence Duplication Levels
 - Overrepresented sequences
 - Adapter Content



FastQC: Per base sequence quality

- Nanopore



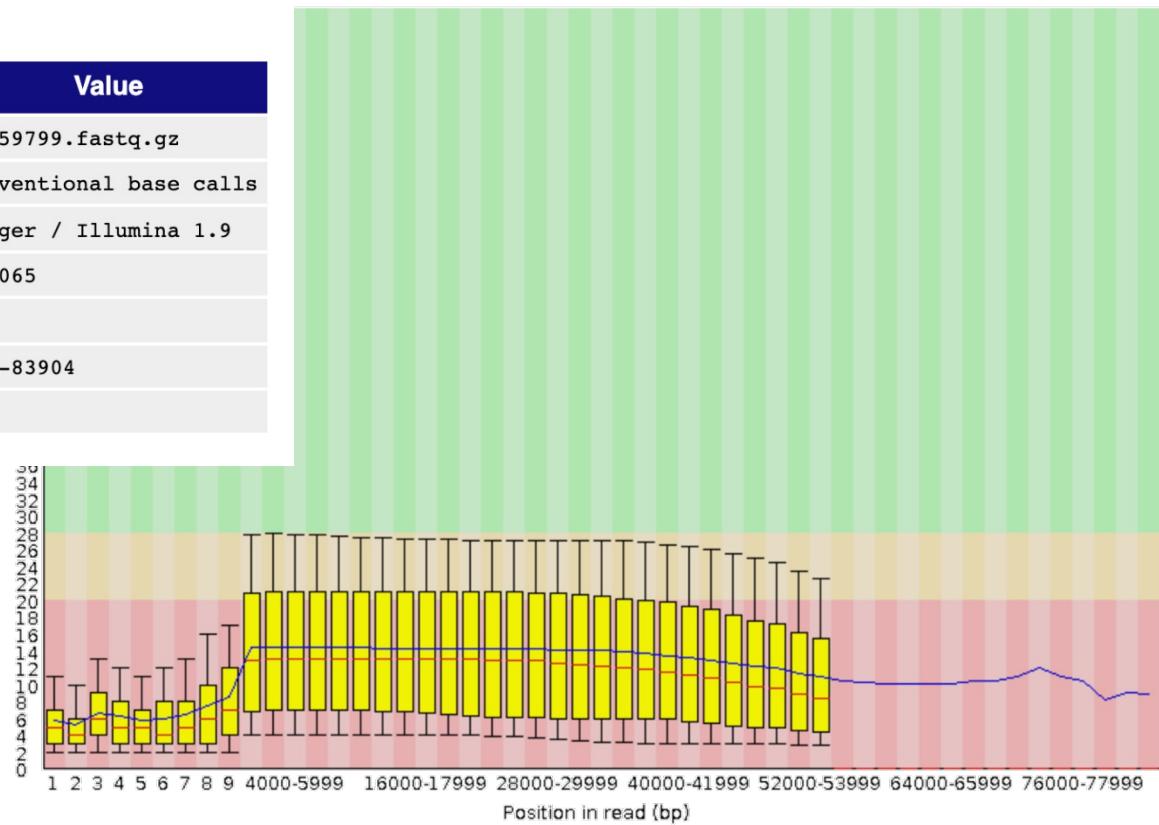
Basic Statistics

Measure	Value
Filename	FAH59799.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	468065
Sequences flagged as poor quality	0
Sequence length	116-83904
%GC	54



Per base sequence quality

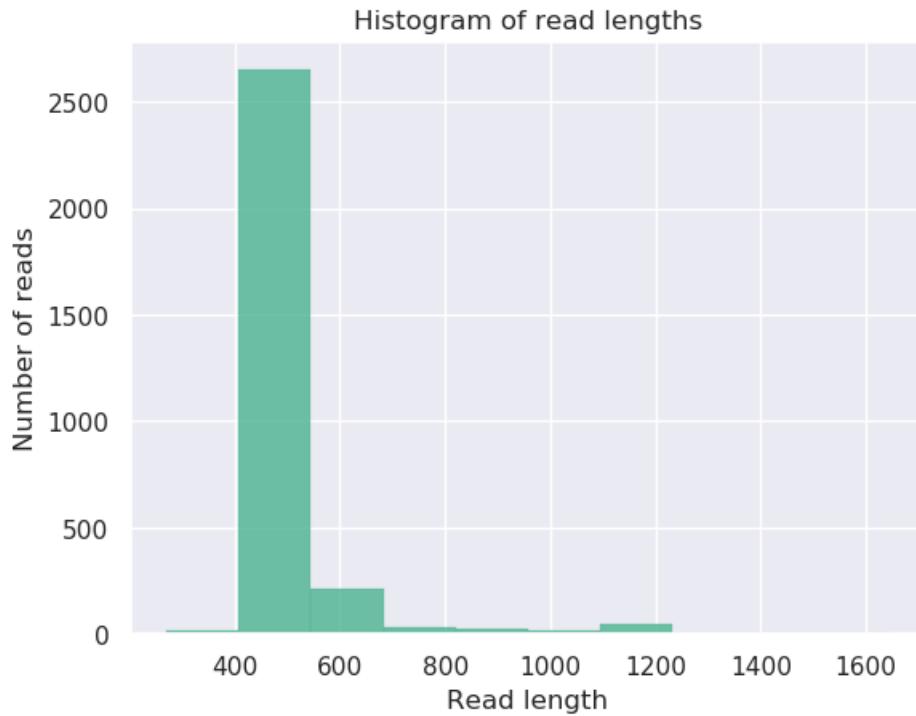
Quality scores across all bases (Sanger / Illumina 1.9 encoding)



Sequencing quality assessment: NanoPlot

- NanoPlot

Histogram of read lengths



NanoPlot report

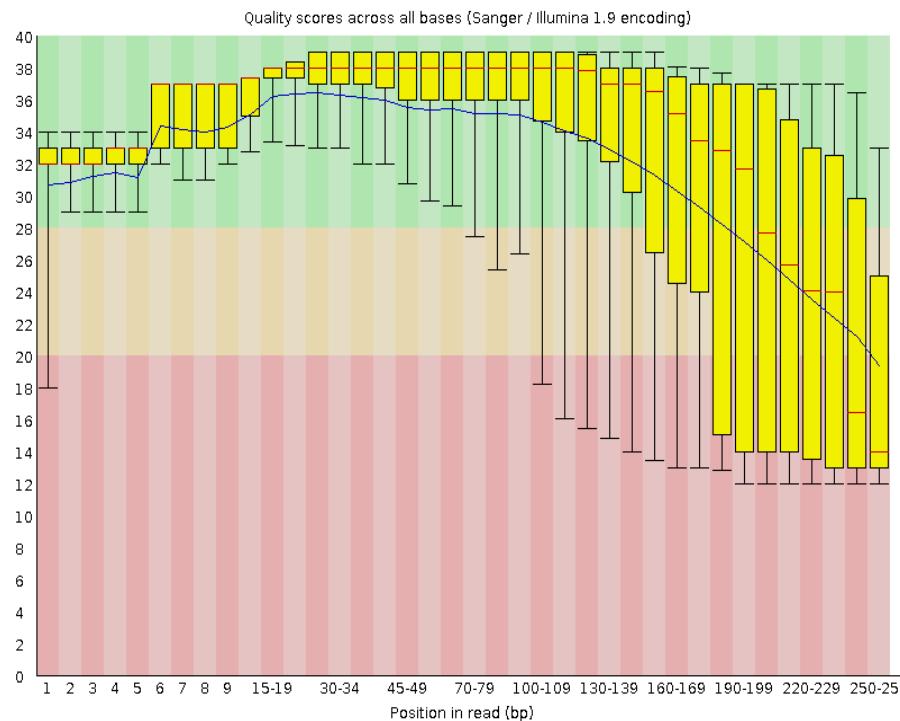
Summary statistics

feature	
General summary	
Mean read length	537.5
Mean read quality	13.9
Median read length	516.0
Median read quality	14.0
Number of reads	3,000.0
Read length N50	517.0
Total bases	1,612,409.0
Number, percentage and megabases of reads above quality cutoffs	
>Q5	3000 (100.0%) 1.6Mb
>Q7	3000 (100.0%) 1.6Mb
>Q10	2865 (95.5%) 1.5Mb
>Q12	2461 (82.0%) 1.3Mb
>Q15	905 (30.2%) 0.5Mb
Top 5 highest mean basecall quality scores and their read lengths	
1	21.3 (504)
2	20.2 (517)
3	20.1 (509)
4	20.0 (526)
5	19.9 (530)
Top 5 longest reads and their mean basecall quality score	
1	1643 (13.5)
2	1641 (16.7)
3	1533 (12.5)
4	1427 (13.2)
5	1383 (15.0)

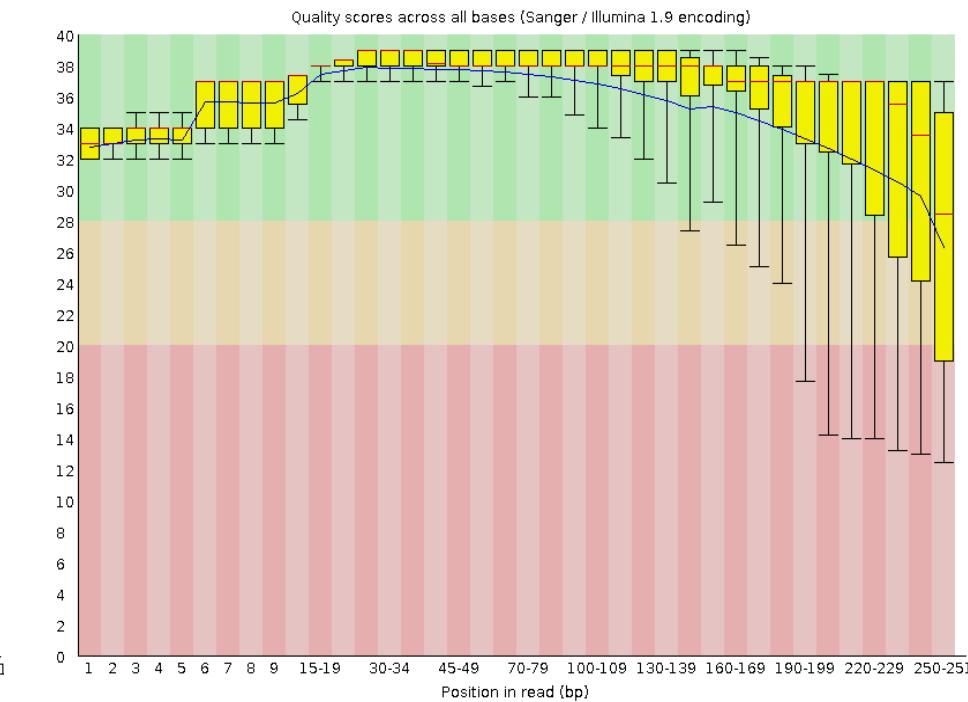
FastQC: Per base sequence quality

- Miseq assymetry

✗ Per base sequence quality



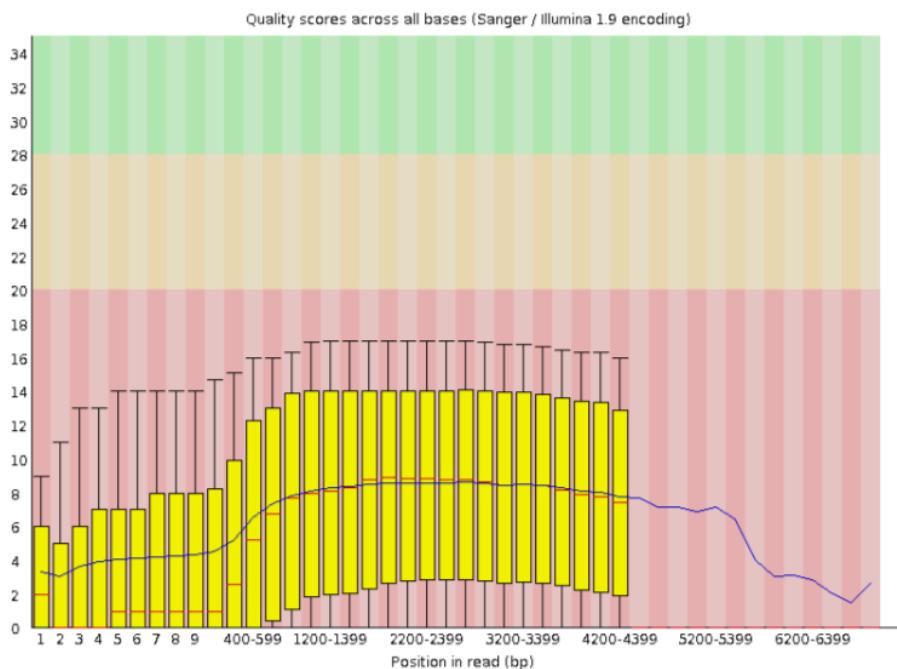
✓ Per base sequence quality



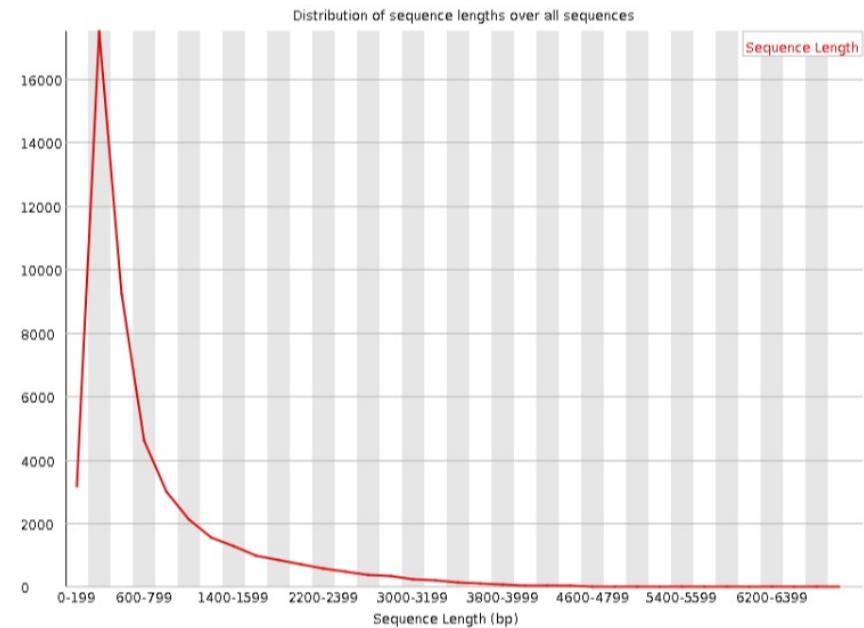
FastQC: Per base sequence quality

- SMRT PacBio

✖ Per base sequence quality

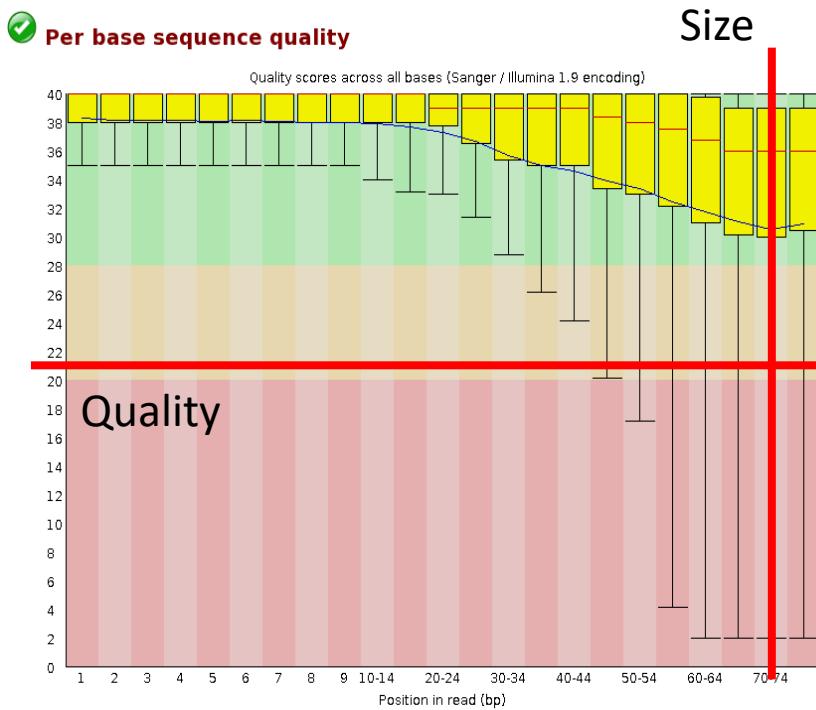


⌚ Sequence Length Distribution



Sequence filtering

- Remove residual adapters
 - Depending on used library
- Filtering parameters
 - Quality filtering
 - Overall mean quality
 - Local mean quality
 - Sequence end
 - Sliding window
 - Size filtering
 - Overall sequence size
 - Remaining sequence size after filtering



Sequencing quality filtering

- Illumina:
 - Fastp
 - Trimmomatic
 - Trim galore!
- Nanopore:
 - Nanofilt
 - ARTIC guppyplex

Sequencing quality filtering: fastp

- Fastp**

fastp report

Summary

General

fastp version:	0.20.1 (https://github.com/OpenGene/fastp)
sequencing:	paired end (149 cycles + 149 cycles)
mean length before filtering:	116bp, 116bp
mean length after filtering:	117bp, 117bp
duplication rate:	1.704150%
Insert size peak:	95
Detected read1 adapter:	CACCTAAGTTGGCGTATAACGCGTAATATATCTGGGTTTCTACAAAATCATACCGTCT
Detected read2 adapter:	CACCTAAGTTGGCGTATAACGCGTAATATATCTGGGTTTCTACAAAATCATACCGTCT

Before filtering

total reads:	1.296756 M
total bases:	151.424921 M
Q20 bases:	143.112834 M (94.510754%)
Q30 bases:	137.905419 M (91.071812%)
GC content:	40.410939%

After filtering

total reads:	854.250000 K
total bases:	100.537720 M
Q20 bases:	99.598139 M (99.065444%)
Q30 bases:	97.968091 M (97.444115%)
GC content:	39.665634%

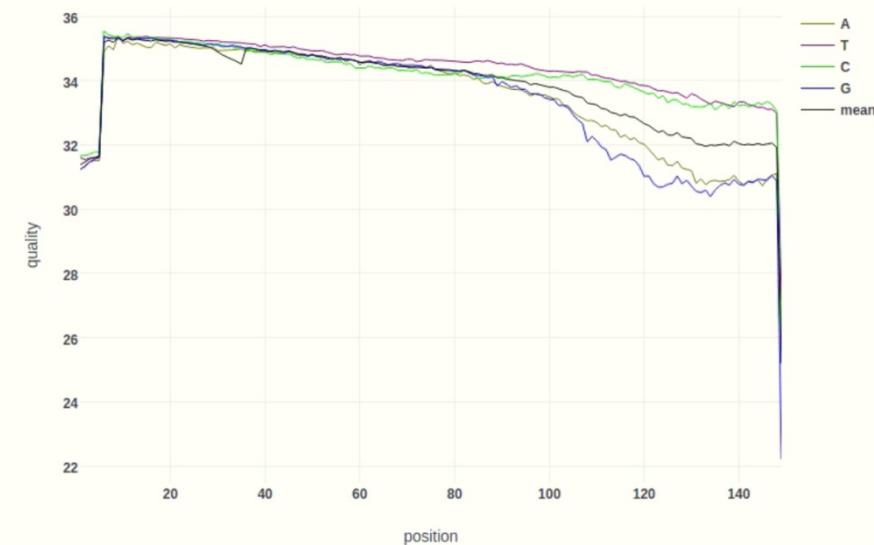
Filtering result

reads passed filters:	854.250000 K (65.875924%)
reads with low quality:	352.272000 K (27.165635%)
reads with too many N:	84 (0.0006478%)
reads too short:	90.150000 K (6.951963%)

Before filtering

Before filtering: read1: quality

Value of each position will be shown on mouse over.

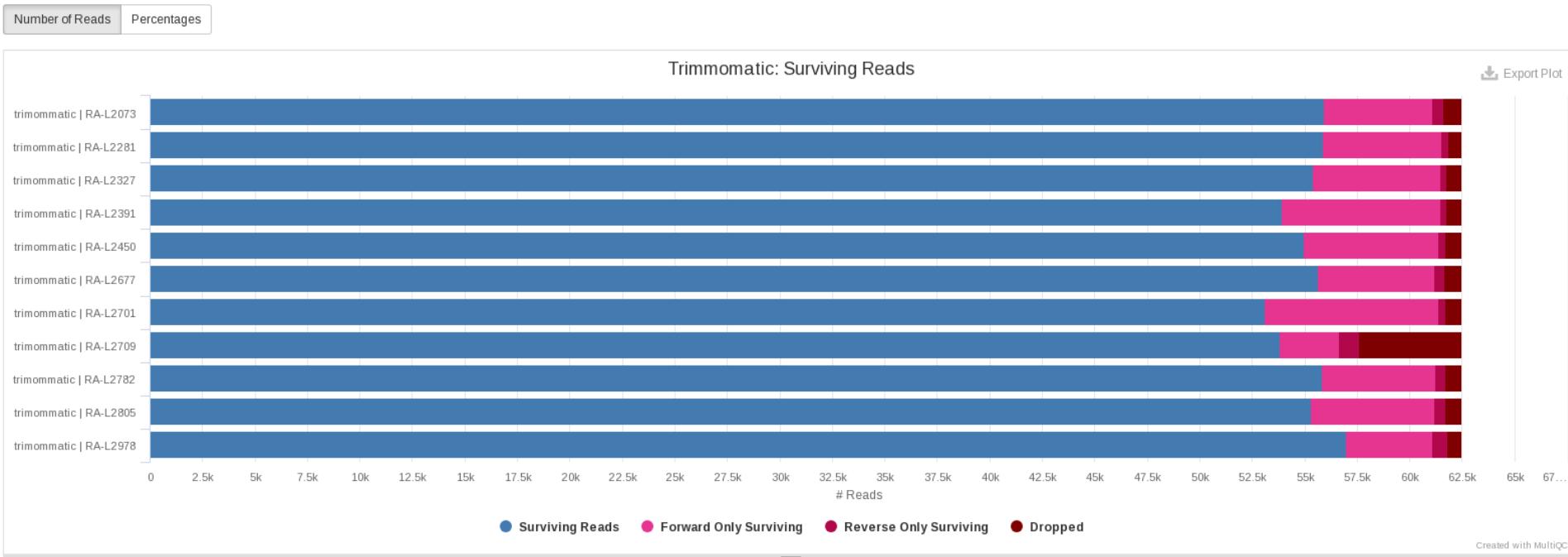


Sequencing quality filtering: Trimmomatic

- Trimmomatic

Trimmomatic

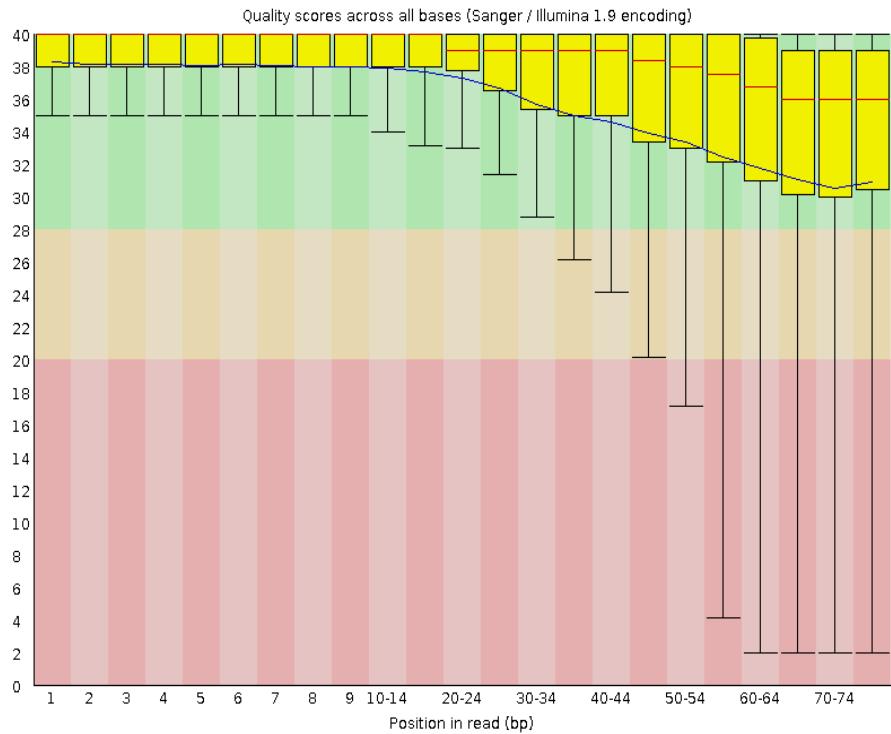
Trimmomatic is a flexible read trimming tool for Illumina NGS data.



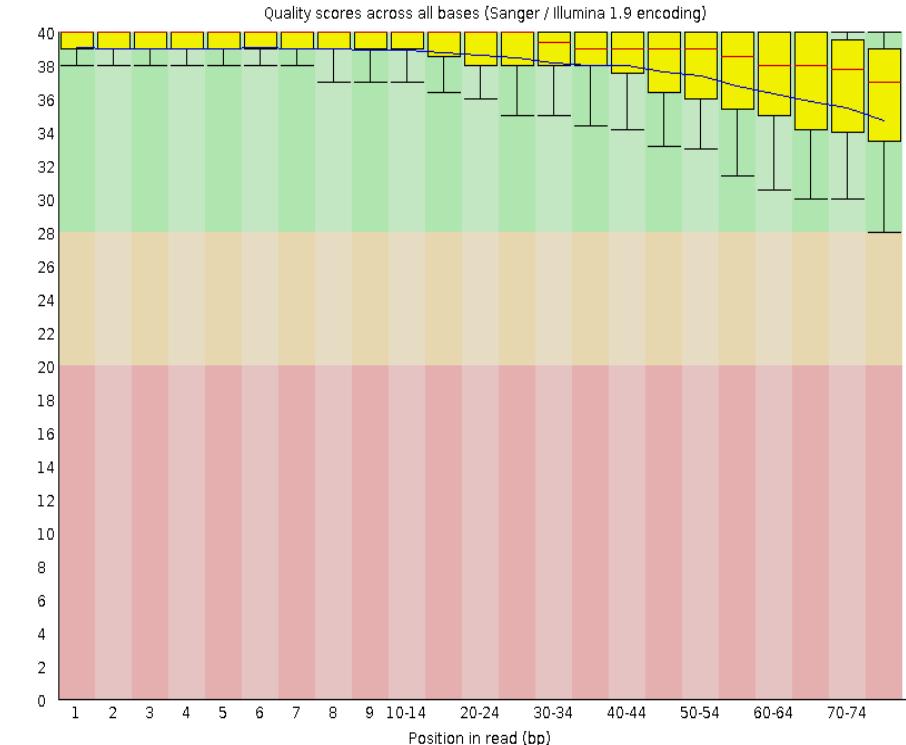
Sequence filtering

- Example of quality filtering

✓ Per base sequence quality



✓ Per base sequence quality



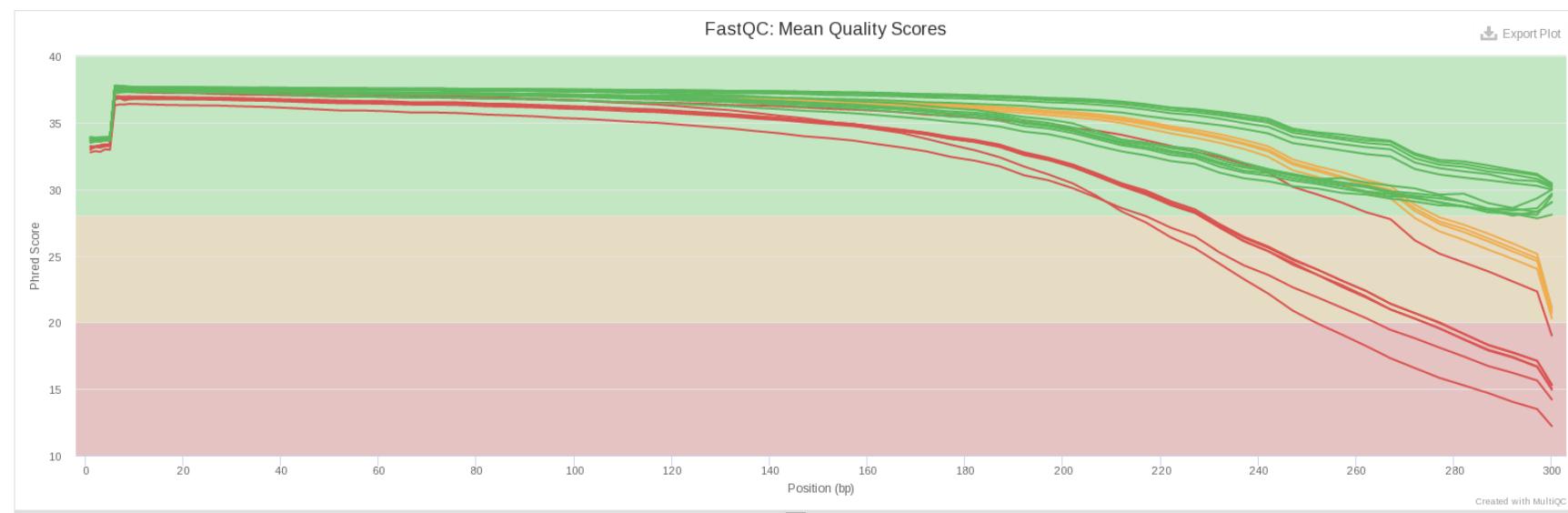
Sequence filtering: stats with MultiQC

Sequence Quality Histograms

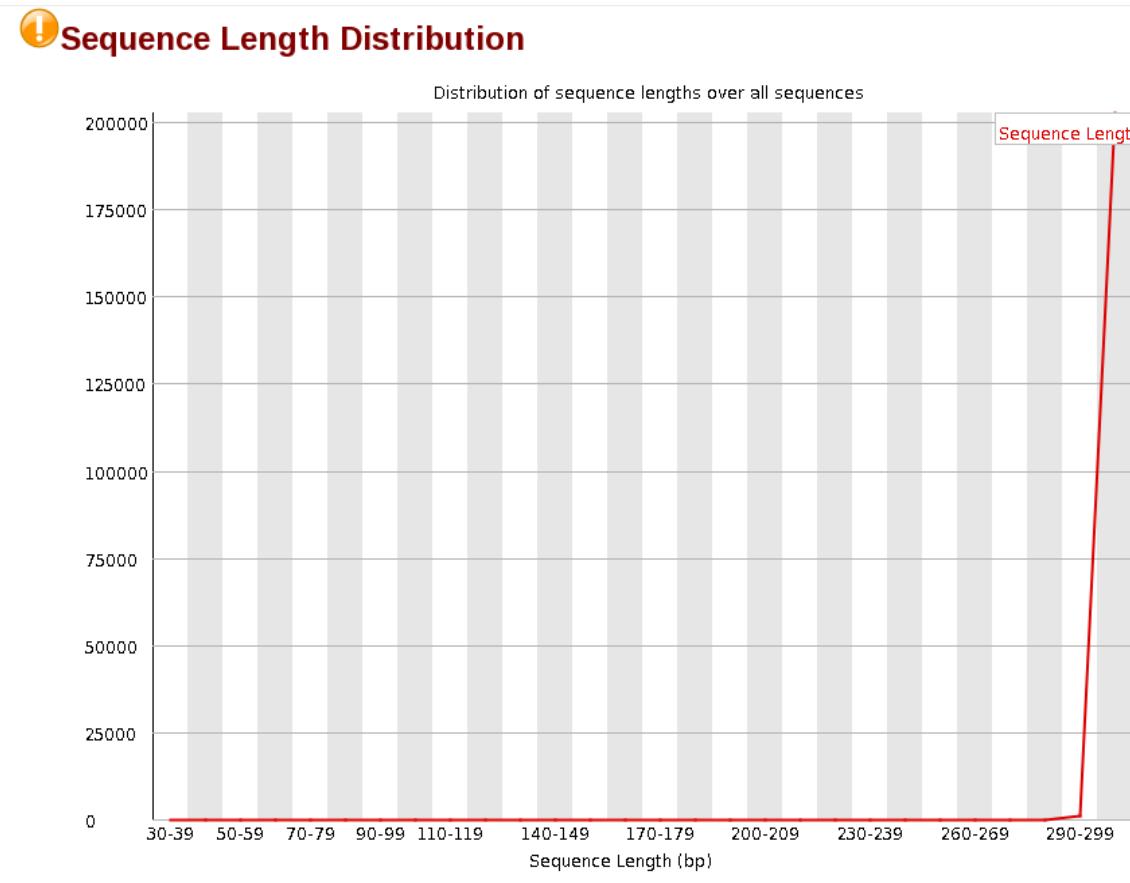
11 4 7

The mean quality value across each base position in the read. See the [FastQC help](#).

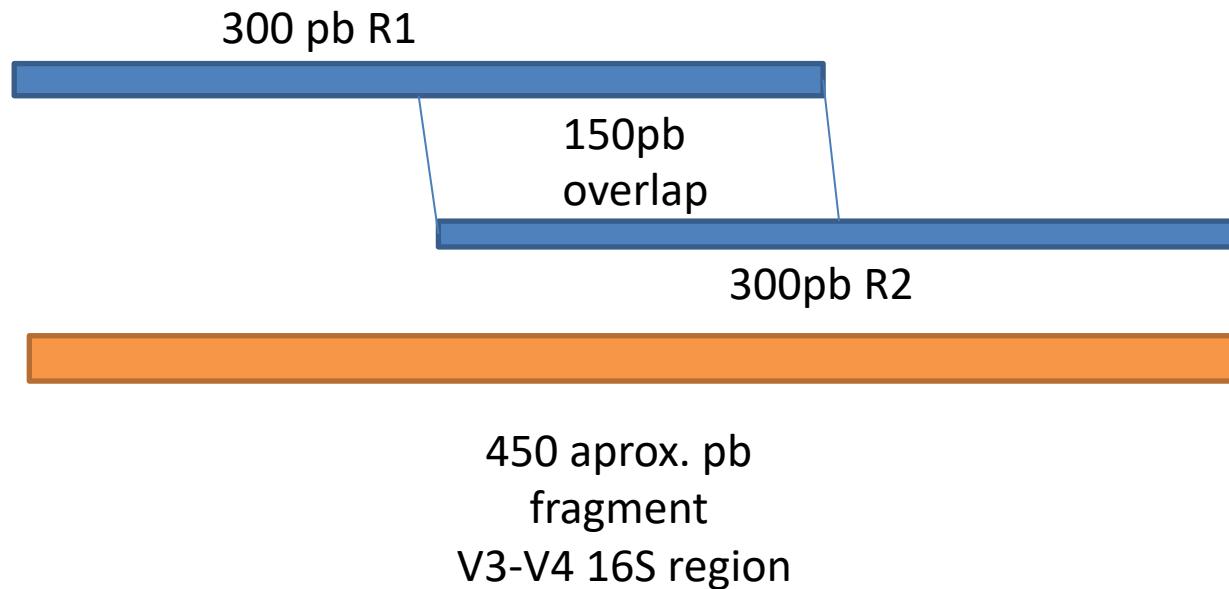
Y-Limits: off



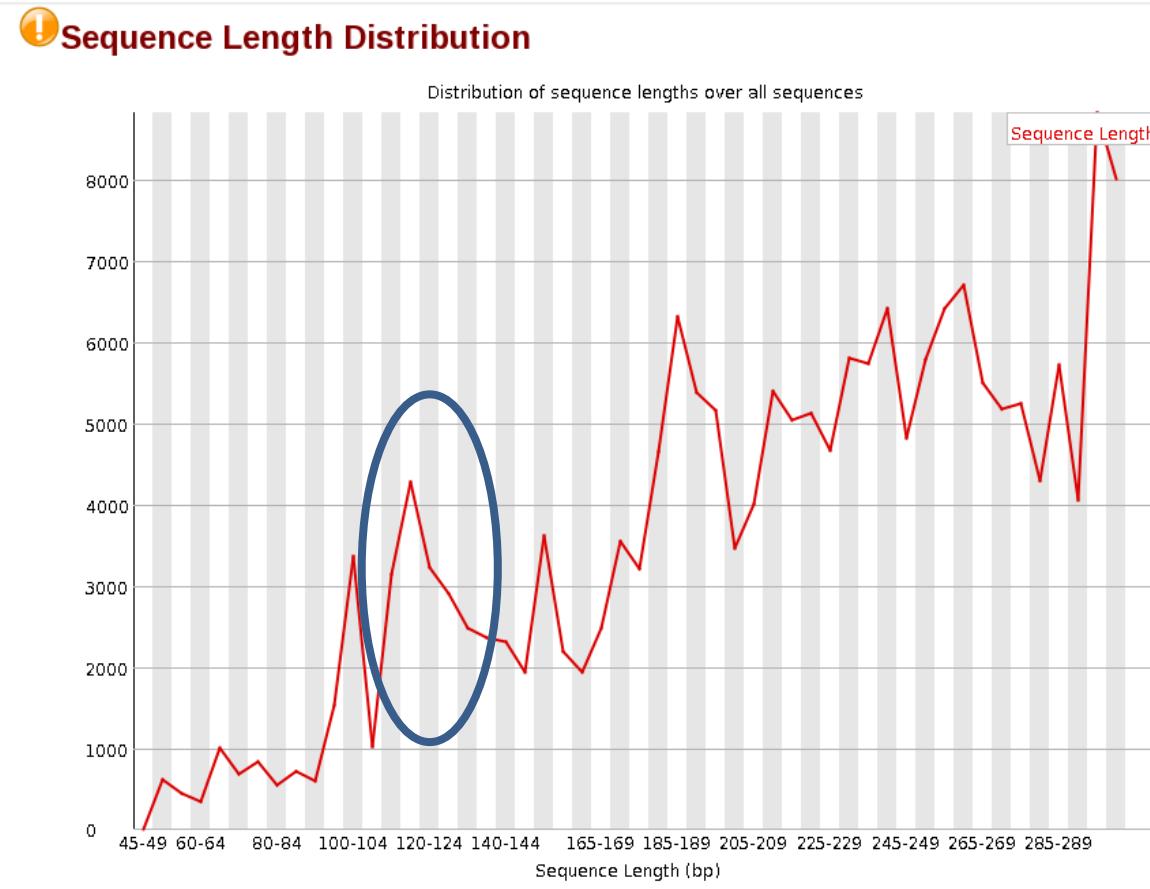
Quality filtering in metagenomic samples



Quality filtering in metagenomic samples

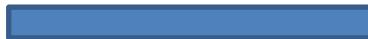


Quality filtering in metagenomic samples



Quality filtering in metagenomic samples

100 pb R1



No overlap



100 pb R2



450 aprox. pb
fragment
V3-V4 16S
region

Questions?