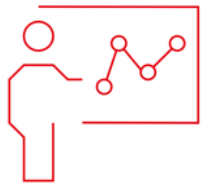
Introduction to RNA-Seq: Quality Control



Wandrille Duchemin

"Raw data": FASTQ format

```
@HWI-ST143:498:D0T4GACXX:7:1101:1474:2111 1:N:0:TGACCA
TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG
+
@@BF#2ADHHHHHHJJJJIJJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ
@HWI-ST143:498:D0T4GACXX:7:1101:1424:2149 1:N:0:TGACCA
CAGATGTTTTGGTAAGAAGAATGCCATTAGCAATTGGTGGAGGAGCAGGA
+
BBCFFFEFHHHHHHJIJIJJHIIJIIJJJJJJJJJJJIJHIJIJJIJIGIIG
```

Paired data: two separate files for forward and reverse with same ordering

"Raw data": FASTQ format

Read ID
line starts with @

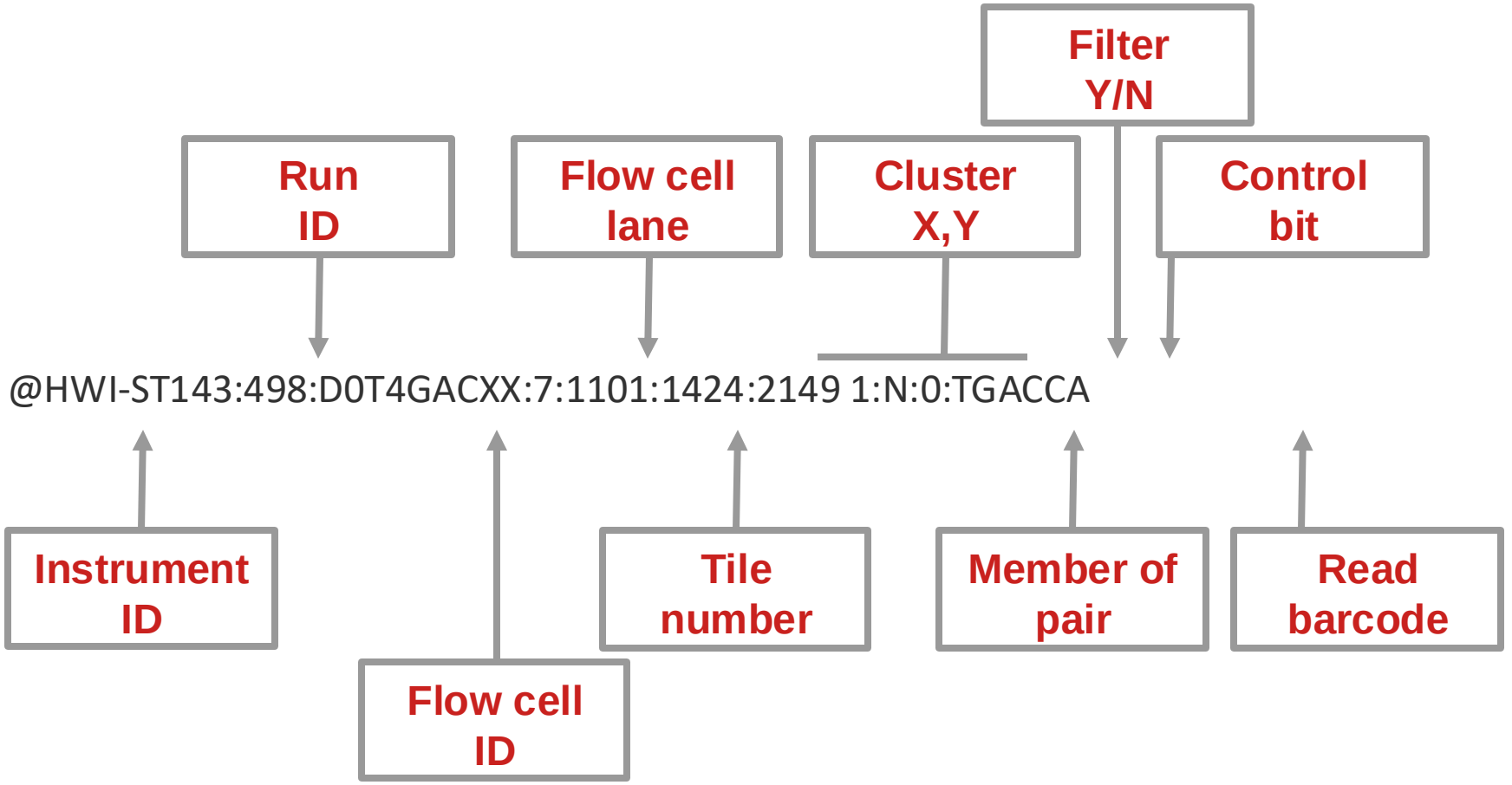
```
@HWI-ST143:498:D0T4GACXX:7:1101:1474:2111 1:N:0:TGACCA
TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG
+
@@BF#2ADHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ
@HWI-ST143:498:D0T4GACXX:7:1101:1424:2149 1:N:0:TGACCA
CAGATGTTTTGGTAAGAAGAATGCCATTAGCAATTGGTGGAGGAGCAGGA
+
BBCFFFEFHHHHHJIJIJJHIIJIIJJJJJJJJJJIJHIJIJJIJIGIIG
```

sequence

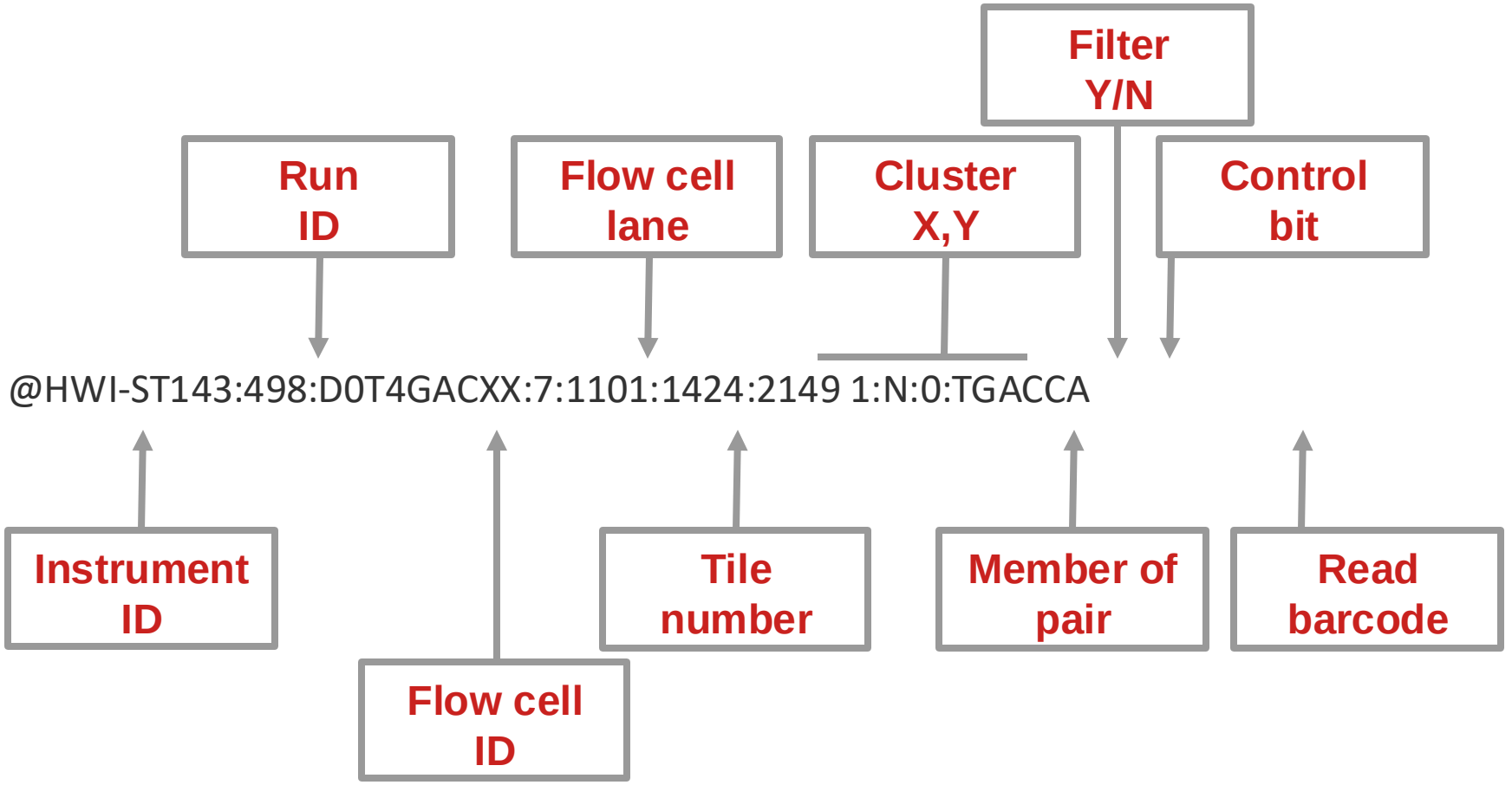
quality line

Paired data: two separate files for forward and reverse with same ordering

"Raw data": FASTQ format - header



"Raw data": FASTQ format - header



Depends on the sequencing technology.
It was changed several times by illumina and others

"Raw data": FASTQ format - quality line

TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG

@@BF#2ADHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ

Each nucleotide is associated to a quality line

"Raw data": FASTQ format - PHRED score

Probability that a base is incorrect (p)

- Quality (Q) = $-10 \log_{10}(p)$

ASCII encoded

P-value	PHRED	Probability of incorrect base call	Base call accuracy
10^{-1}	10	1/10	90%
10^{-2}	20	1/100	99%
10^{-3}	30	1/1000	99.9%
10^{-4}	40	1/10'000	99.99%

"Raw data": FASTQ format - quality line

TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG

@@BF#2ADHHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ

Illumina v1.8 and later (ASCII_BASE=33)

Q	ASCII	P	Q	ASCII	P	Q	ASCII	P	Q	ASCII	P
1	"	0.79433	12	-	0.06310	23	8	0.00501	34	C	0.00040
2	#	0.63096	13	.	0.05012	24	9	0.00398	35	D	0.00032
3	$	0.50119	14	/	0.03981	25	:	0.00316	36	E	0.00025
4	%	0.39811	15	0	0.03162	26	;	0.00251	37	F	0.00020
5	&	0.31623	16	1	0.02512	27	<	0.00200	38	G	0.00016
6	'	0.25119	17	2	0.01995	28	=	0.00158	39	H	0.00013
7	(	0.19953	18	3	0.01585	29	>	0.00126	40	I	0.00010
8	)	0.15849	19	4	0.01259	30	?	0.00100	41	J	0.00008
9	*	0.12589	20	5	0.01000	31	@	0.00079			
10	+	0.10000	21	6	0.00794	32	A	0.00063			
11	,	0.07943	22	7	0.00631	33	B	0.00050			

"Raw data": FASTQ format - quality line

TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG

@@BF#2ADHHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ

Illumina v1.8 and later (ASCII_BASE=33)

Q	ASCII	P	Q	ASCII	P	Q	ASCII	P	Q	ASCII	P
1	"	0.79433	12	-	0.06310	23	8	0.00501	34	C	0.00040
2	#	0.63096	13	.	0.05012	24	9	0.00398	35	D	0.00032
3	$	0.50119	14	/	0.03981	25	:	0.00316	36	E	0.00025
4	%	0.39811	15	0	0.03162	26	;	0.00251	37	F	0.00020
5	&	0.31623	16	1	0.02512	27	<	0.00200	38	G	0.00016
6	'	0.25119	17	2	0.01995	28	=	0.00158	39	H	0.00013
7	(	0.19953	18	3	0.01585	29	>	0.00126	40	I	0.00010
8	)	0.15849	19	4	0.01259	30	?	0.00100	41	J	0.00008
9	*	0.12589	20	5	0.01000	31	@	0.00079			
10	+	0.10000	21	6	0.00794	32	A	0.00063			
11	,	0.07943	22	7	0.00631	33	B	0.00050			

"Raw data": FASTQ format - PHRED +33/+64

Sanger, Illumina v1.3 to 1.7 (ASCII_BASE=64)

Q	ASCII	P	Q	ASCII	P	Q	ASCII	P	Q	ASCII	P
1	A	0.79433	12	L	0.06310	23	W	0.00501	34	b	0.00040
2	B	0.63096	13	M	0.05012	24	X	0.00398	35	c	0.00032
3	C	0.50119	14	N	0.03981	25	Y	0.00316	36	d	0.00025
4	D	0.39811	15	O	0.03162	26	Z	0.00251	37	e	0.00020
5	E	0.31623	16	P	0.02512	27	[	0.00200	38	f	0.00016
6	F	0.25119	17	Q	0.01995	28	\	0.00158	39	g	0.00013
7	G	0.19953	18	R	0.01585	29	]	0.00126	40	h	0.00010
8	H	0.15849	19	S	0.01259	30	^	0.00100			
9	I	0.12589	20	T	0.01000	31	_	0.00079			
10	J	0.10000	21	U	0.00794	32	`	0.00063			
11	K	0.07943	22	V	0.00631	33	a	0.00050			

Illumina v1.8 and later (ASCII_BASE=33)

Q	ASCII	P	Q	ASCII	P	Q	ASCII	P	Q	ASCII	P
1	"	0.79433	12	-	0.06310	23	8	0.00501	34	C	0.00040
2	#	0.63096	13	.	0.05012	24	9	0.00398	35	D	0.00032
3	$	0.50119	14	/	0.03981	25	:	0.00316	36	E	0.00025
4	%	0.39811	15	0	0.03162	26	;	0.00251	37	F	0.00020
5	&	0.31623	16	1	0.02512	27	<	0.00200	38	G	0.00016
6	'	0.25119	17	2	0.01995	28	=	0.00158	39	H	0.00013
7	(	0.19953	18	3	0.01585	29	>	0.00126	40	I	0.00010
8	)	0.15849	19	4	0.01259	30	?	0.00100	41	J	0.00008
9	*	0.12589	20	5	0.01000	31	@	0.00079			
10	+	0.10000	21	6	0.00794	32	A	0.00063			
11	,	0.07943	22	7	0.00631	33	B	0.00050			

Quality Control of FASTQ files with fastqQC

Helps spot problems in the sequencer or in starting library material

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

fastqc generates an html report :

- Average quality per position
- GC% profile
- Adapter presence
- …

Input formats: fastq (gzip), sam, bam

Combining multiple reports: multiQC

- fastQC: 1 report for each fastq file
- MultiQC: combines individual reports in a single file

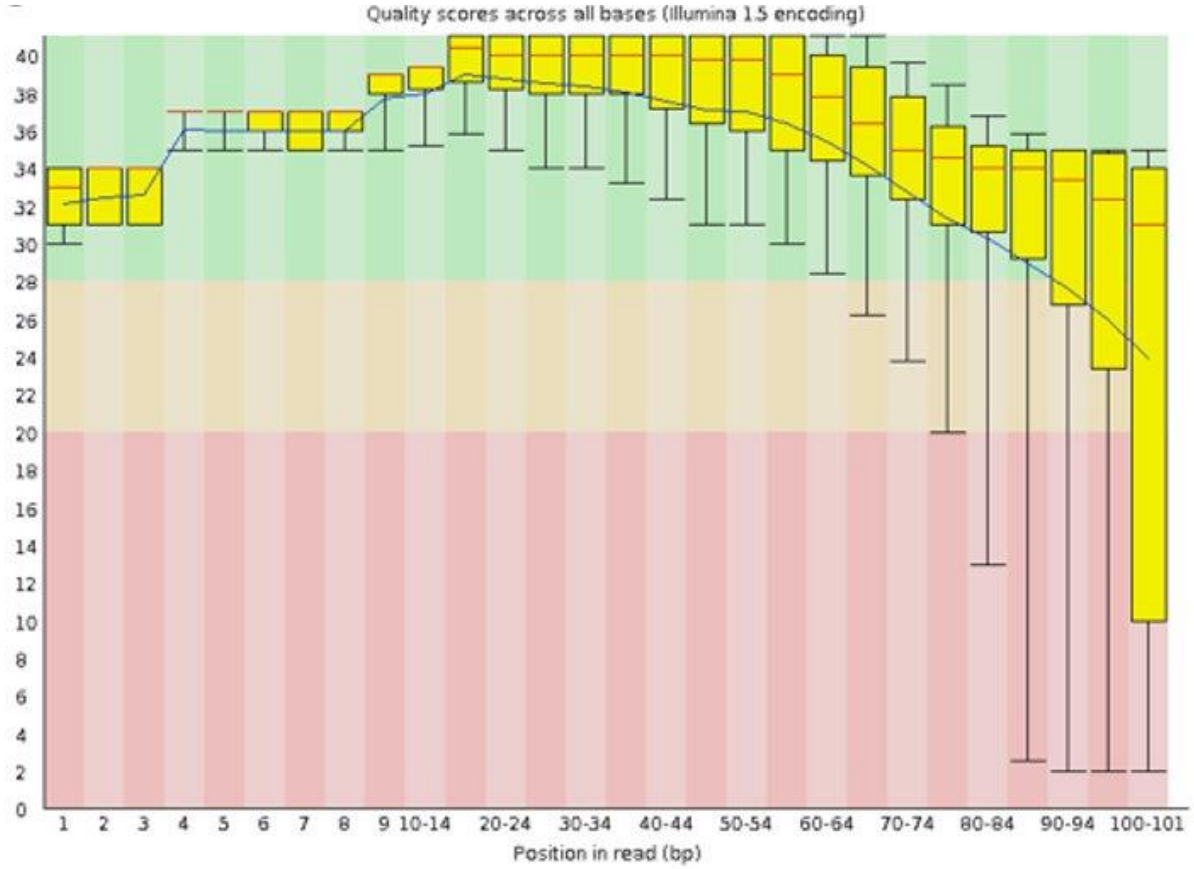
https://www.multiqc.info

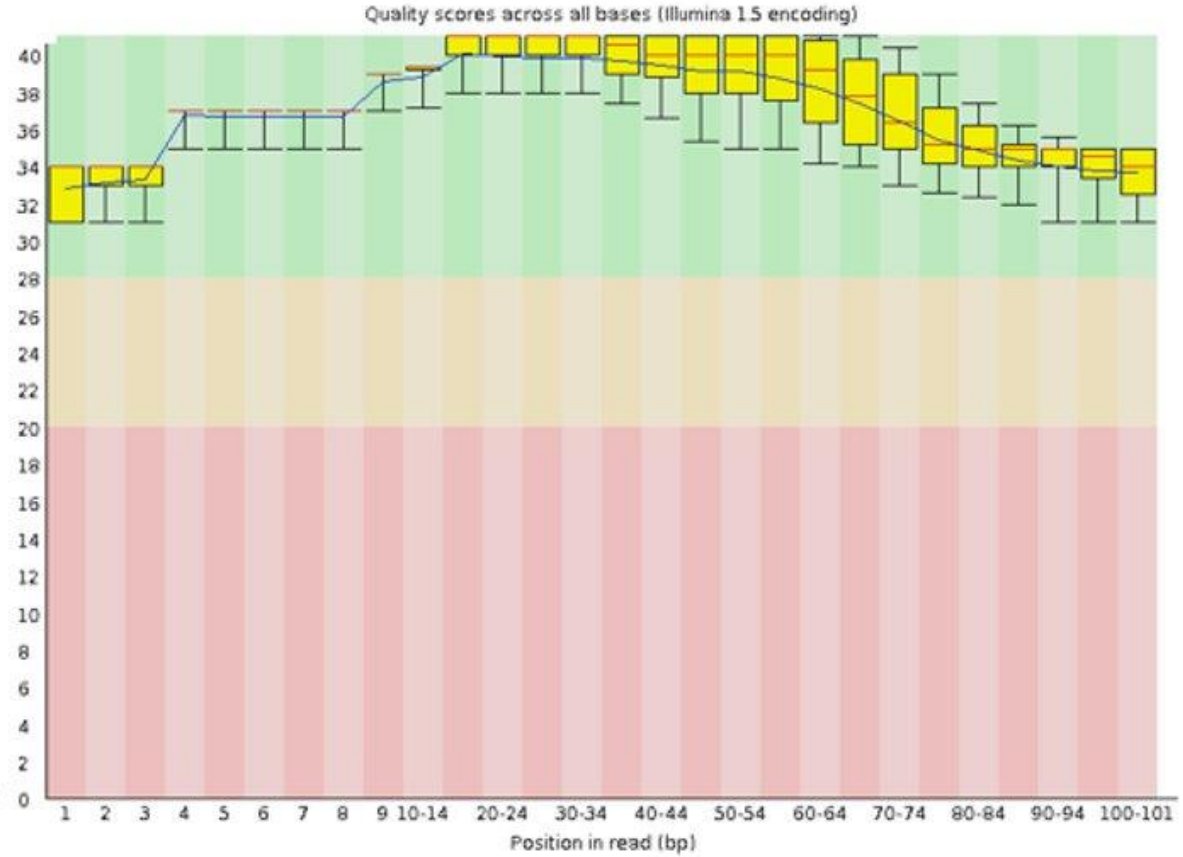
MultiQC also works with other tools outputs:

- Trimming outputs
- Mapping outputs
- …

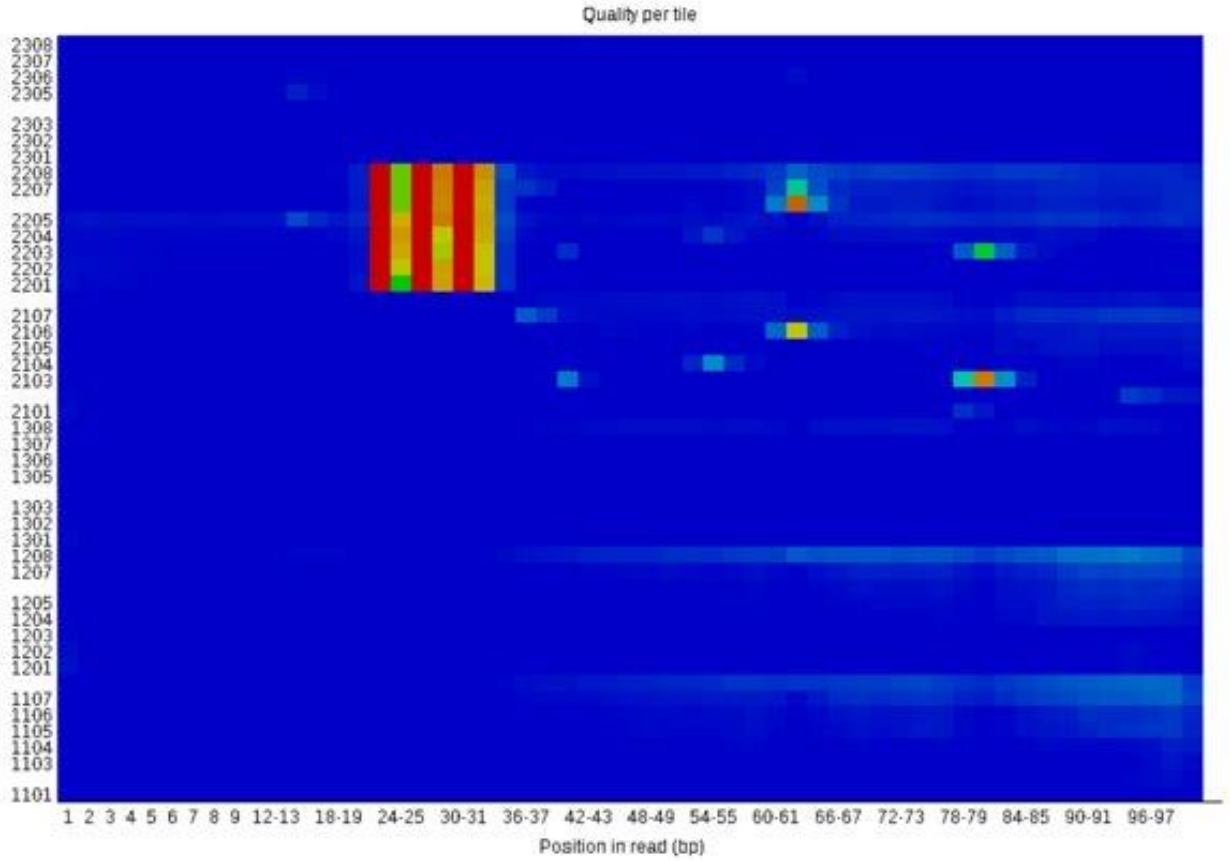
Practical

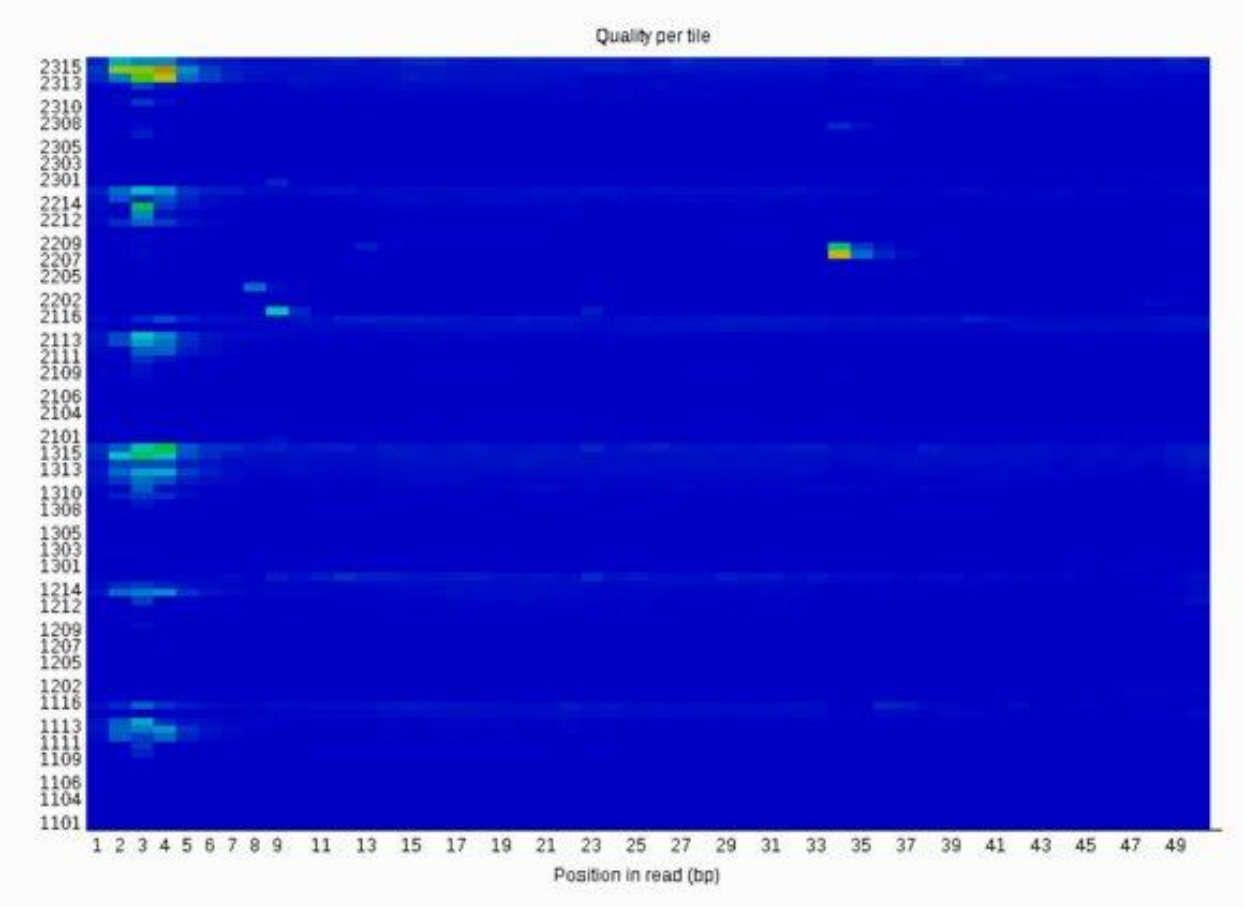
Per base sequence quality





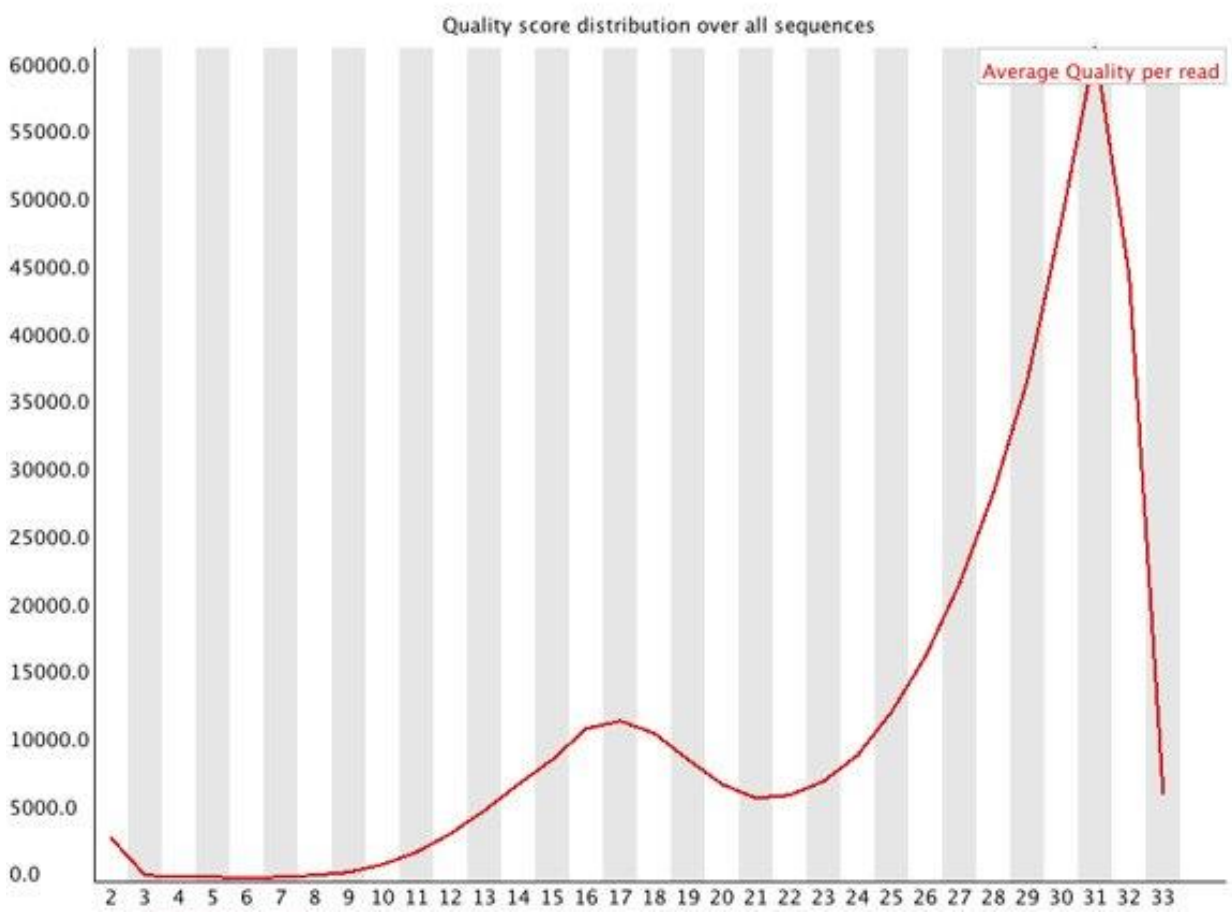
Per tile sequence quality

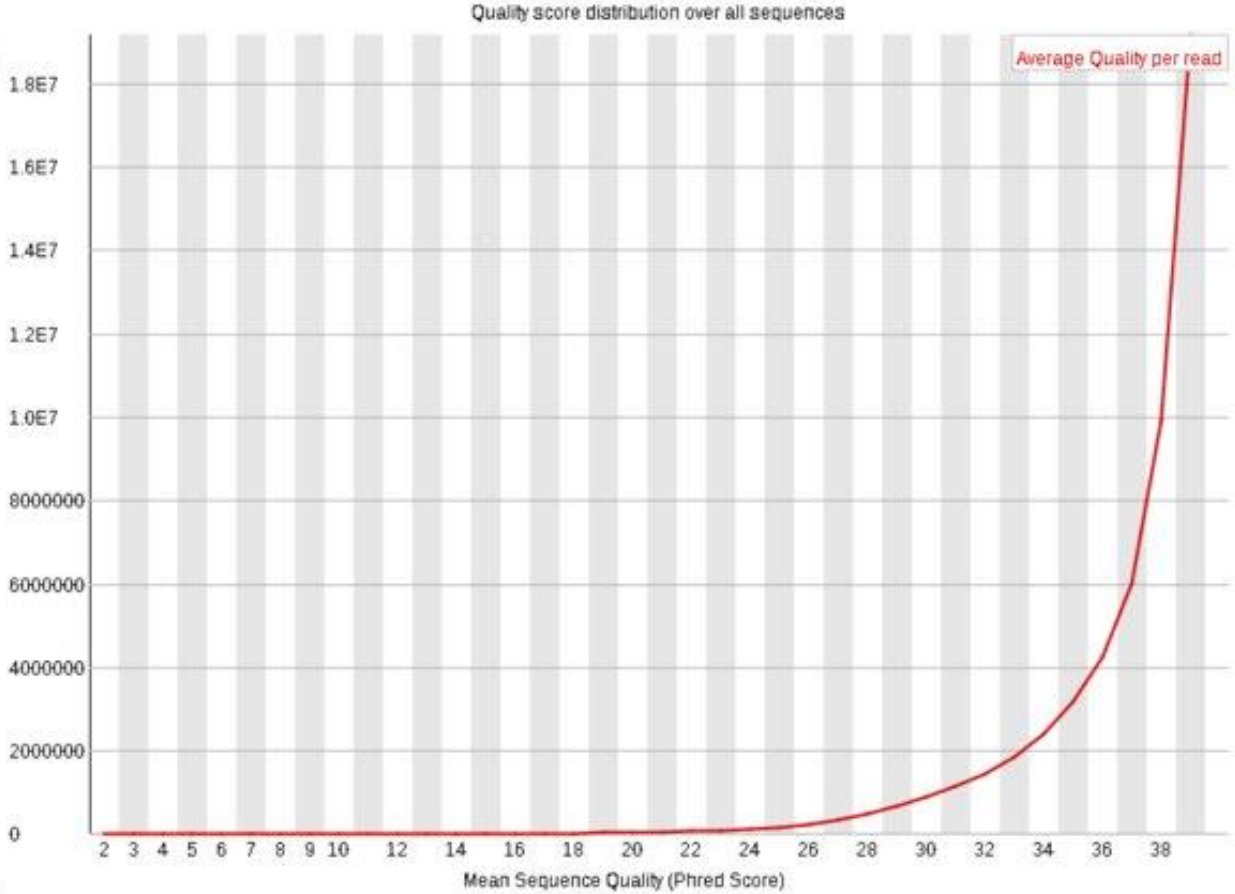




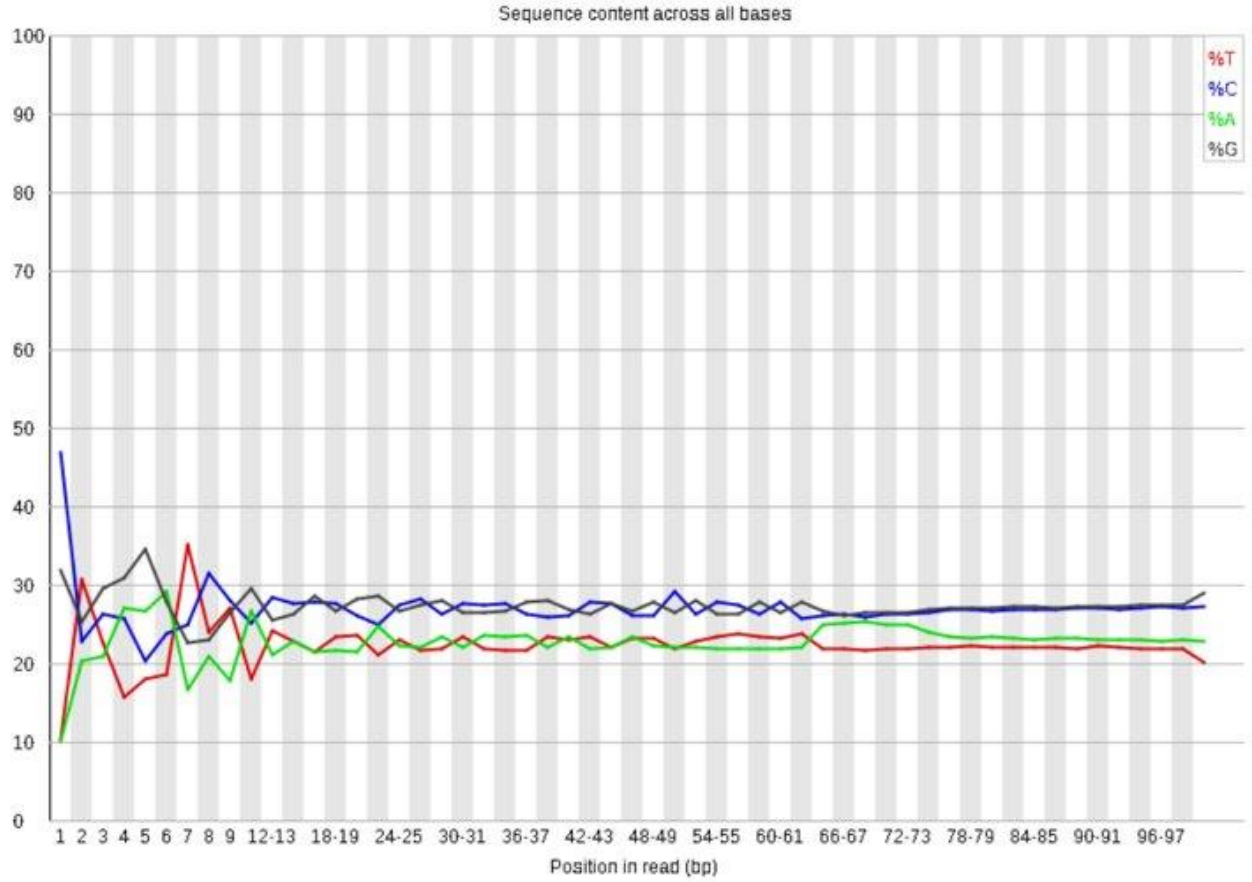
Only present when the fastq id contains the tile id

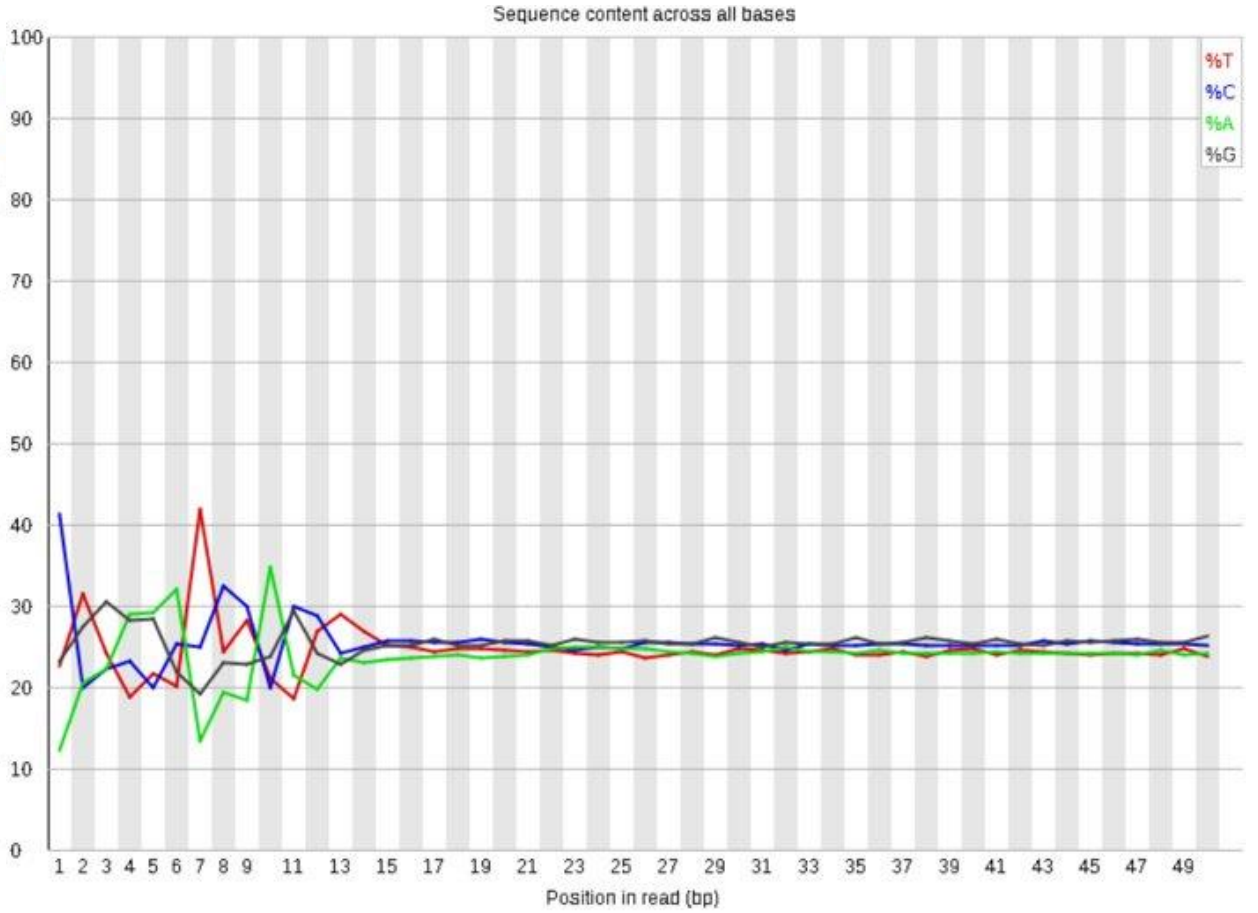
Per sequence quality score



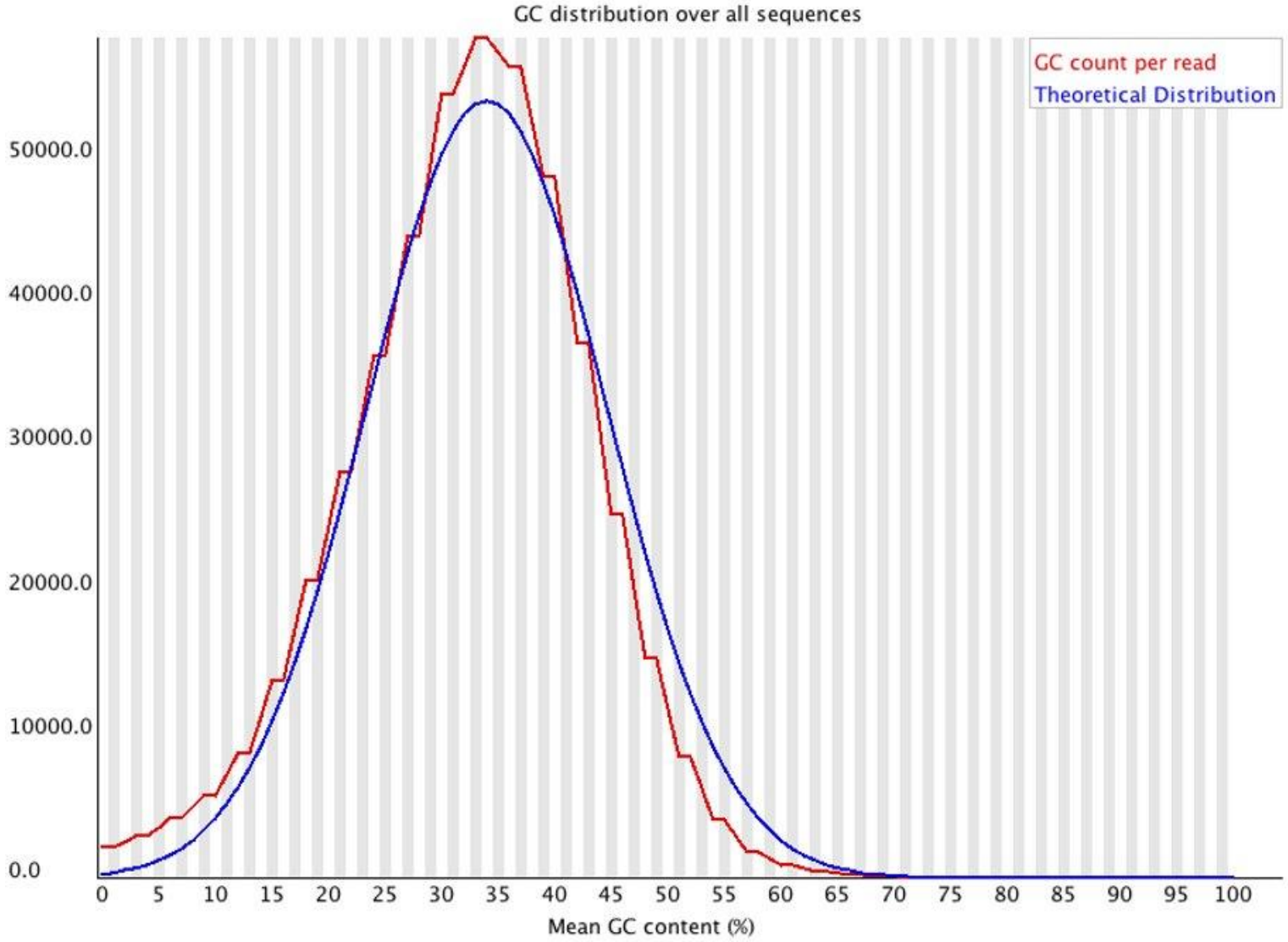


Per base sequence content

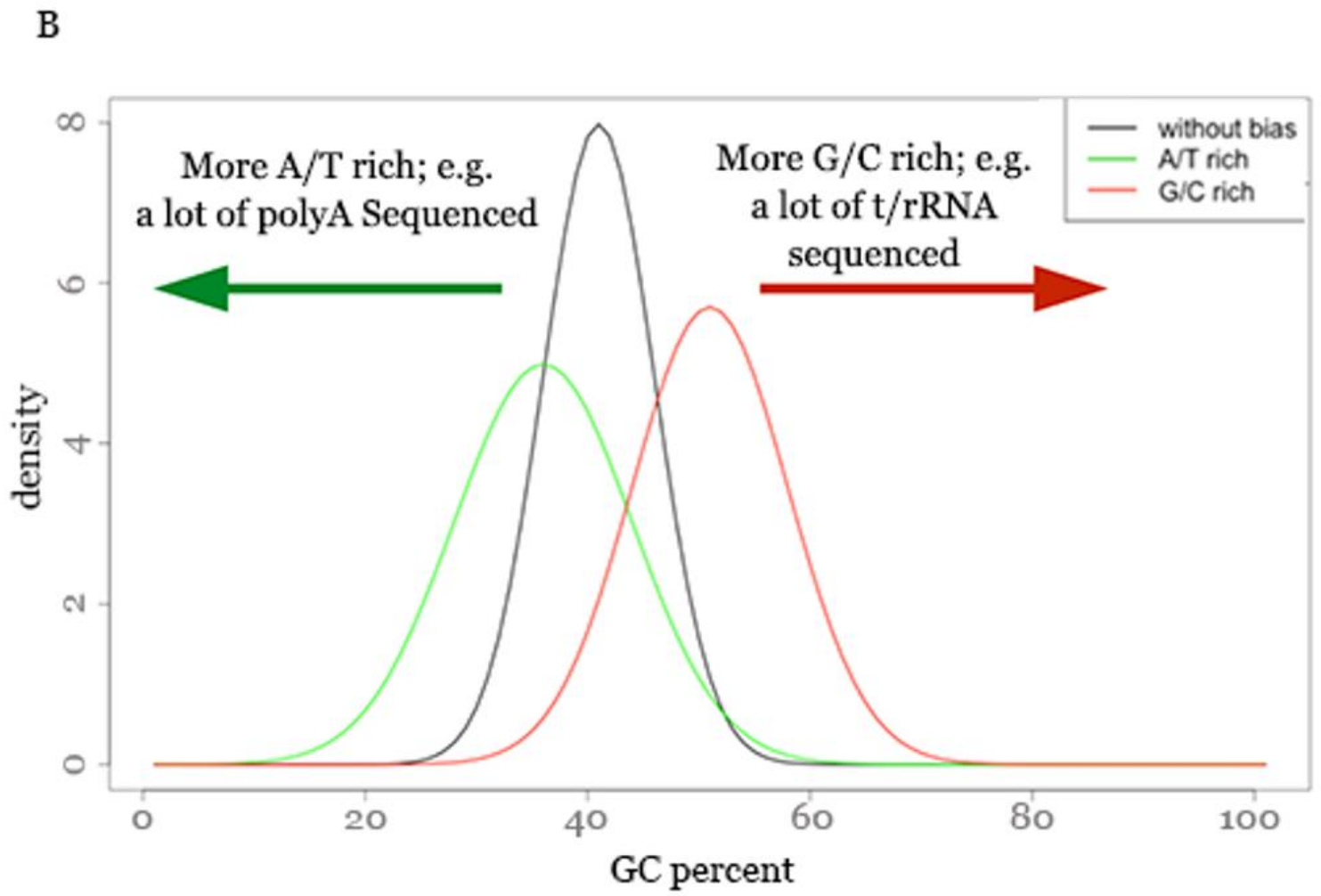




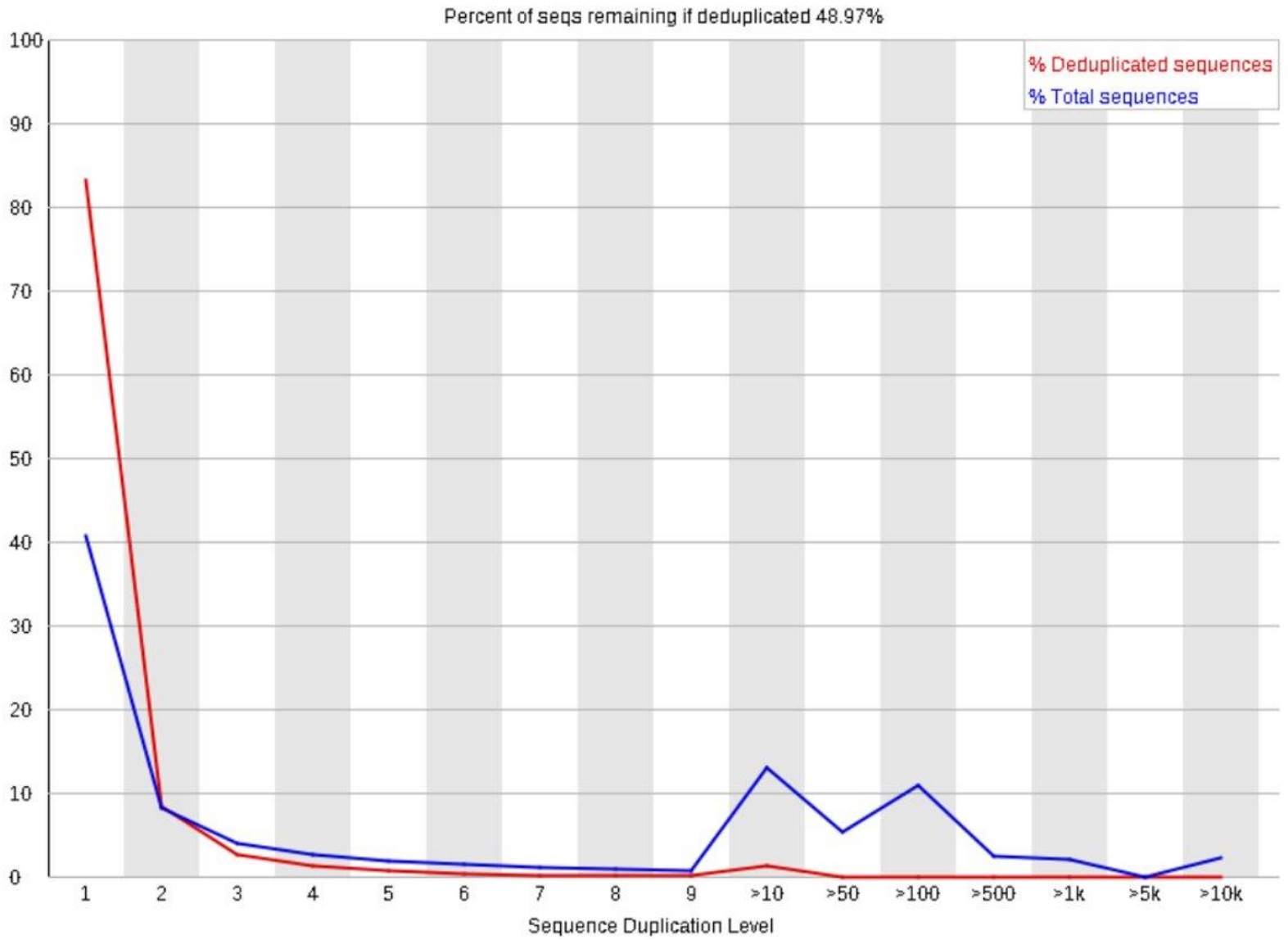
Per sequence GC content



Per sequence GC content



Duplicate sequences



Over-represented sequences

Sequence	Count	Percentage	Possible Source
GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGC	355643	2.113348167370486	TruSeq Adapter, Index 5 (100% over 50bp)
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATG	42318	0.2514675327414971	TruSeq Adapter, Index 5 (100% over 49bp)