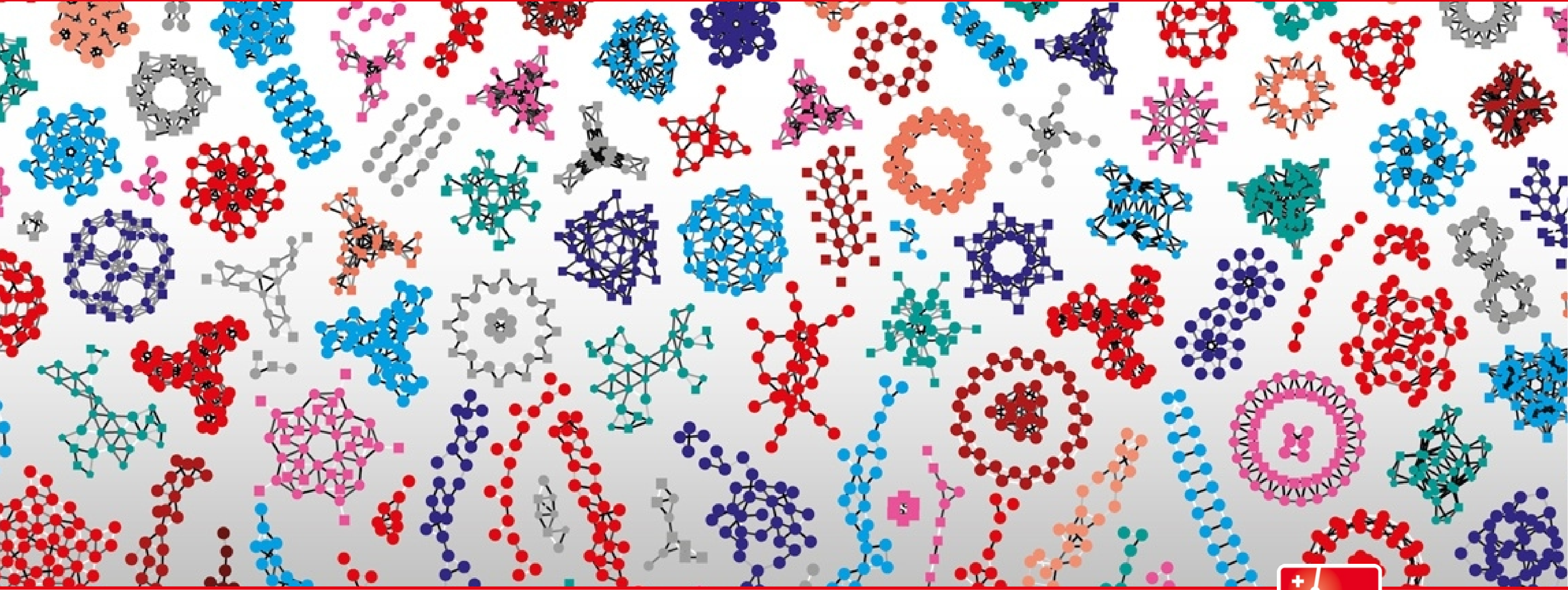


Swiss Institute of Bioinformatics

Introduction to RNA-Seq – Quality Control

Wandrille Duchemin

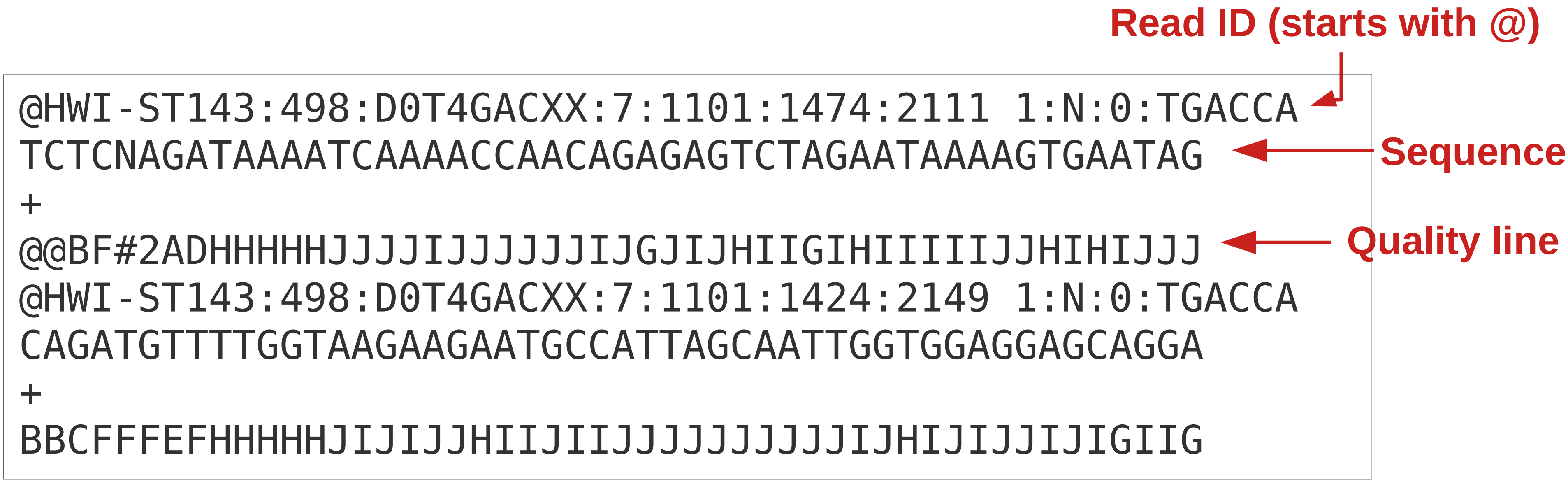
elixir SWITZERLAND

”Raw data”: FASTQ format

```
@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
```

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

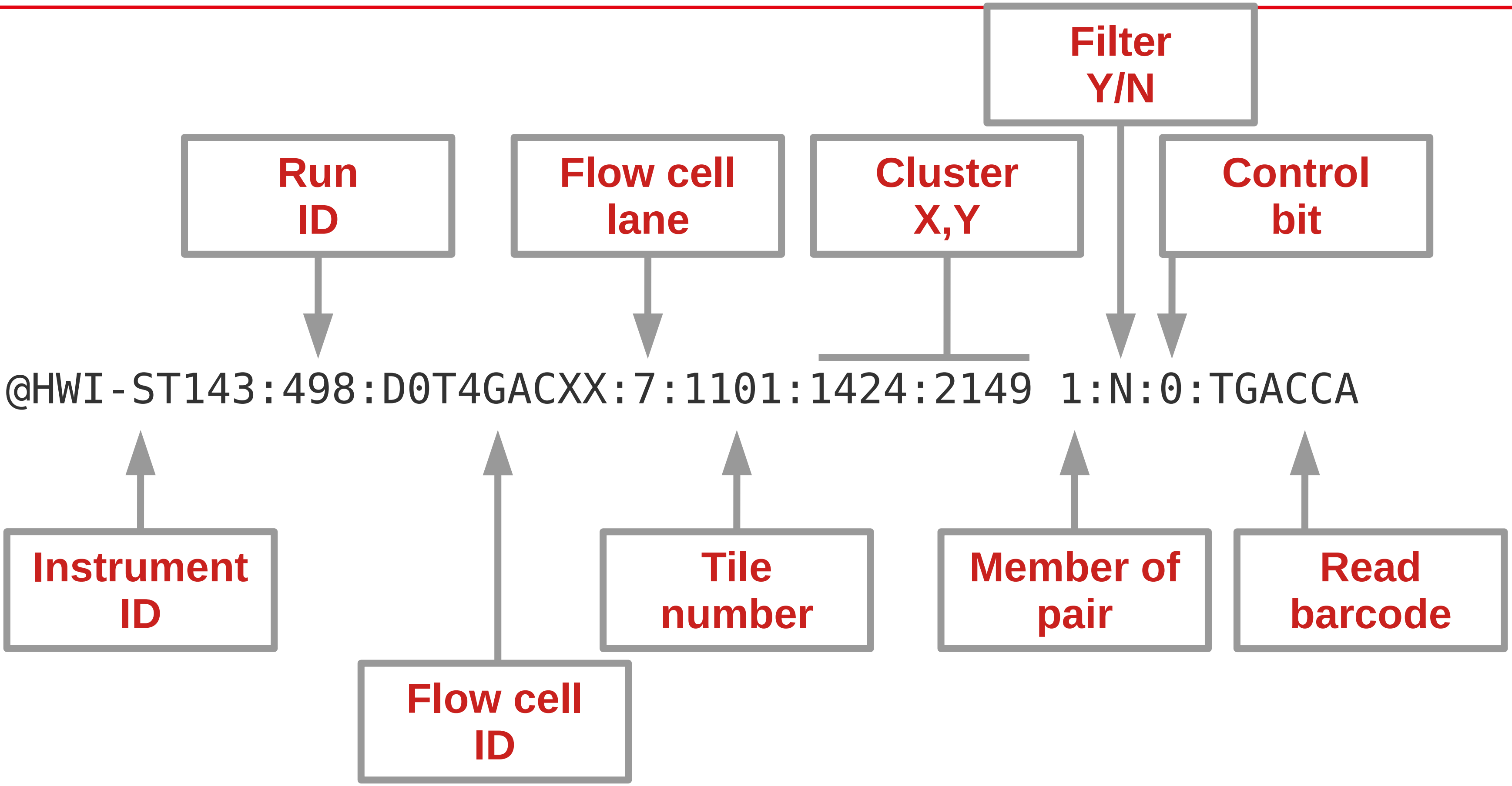
”Raw data”: FASTQ format



```
@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
```

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

”Raw data”: FASTQ format - header



Filter Y/N

Run ID

Flow cell lane

Cluster X,Y

Control bit

```
@HWI-ST143:498:D0T4GACXX:7:1101:1424:2149 1:N:0:TGACCA
```

Instrument ID

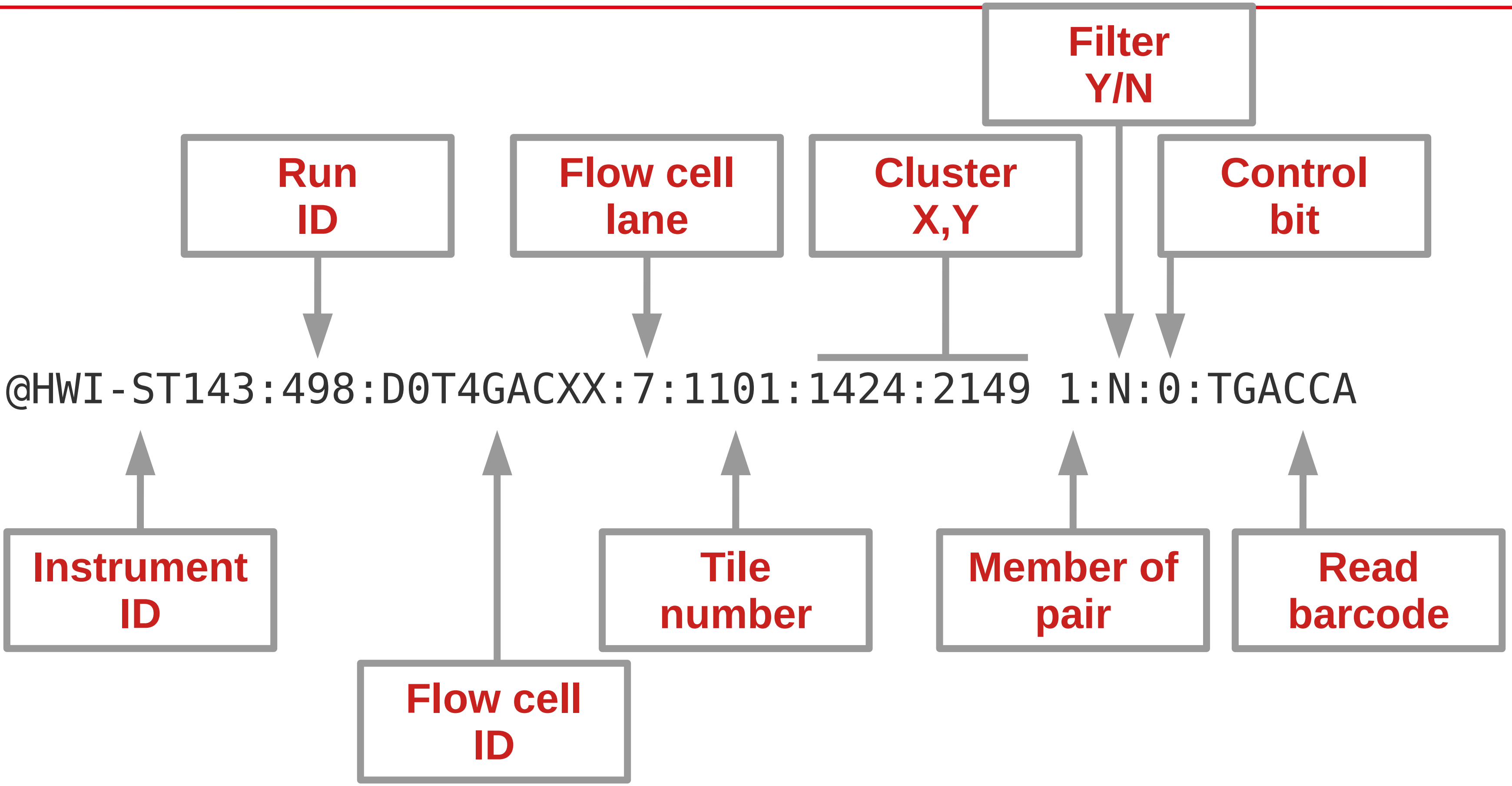
Tile number

Member of pair

Read barcode

Flow cell ID

”Raw data”: FASTQ format - header



- **Depends on sequencing technology used, changed several times by Illumina and others**

”Raw data”: FASTQ format - quality line

```
TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG

@@BF#2ADHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ
```

- **Each nucleotide is associated to a quality value**

”Raw data”: FASTQ format - Phred score

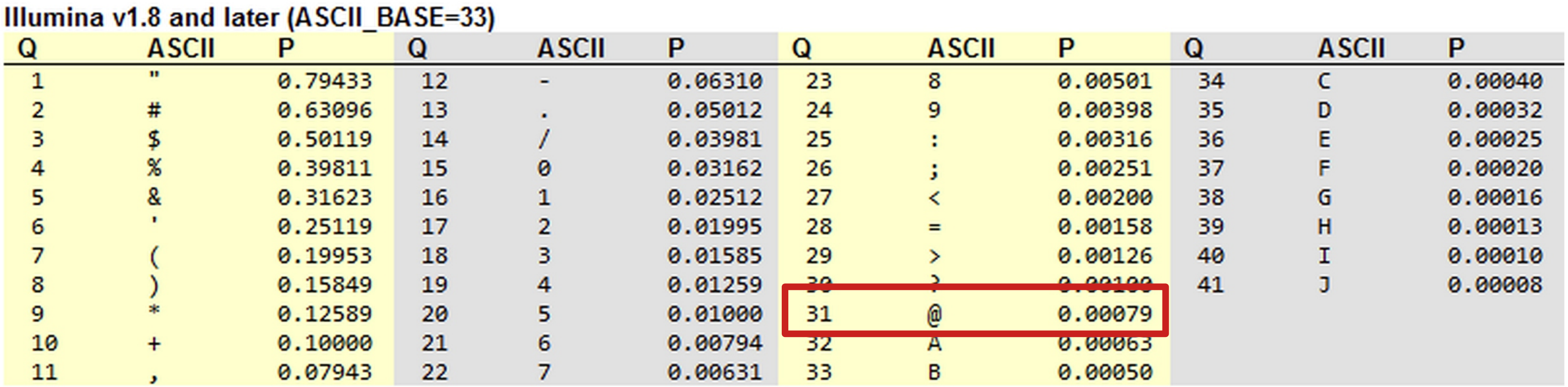
- **ASCII encoded**
- **Represents probability (p) that base call is incorrect**
  - Quality (Q) = -10 $\log_{10}(p)$

P-value	**Phred**	**Probability of incorrect base call**	**Base call accuracy**
1E-01	10	1 in 10	90%
1E-02	20	1 in 100	99%
1E-03	30	1 in 1000	99.9%
1E-04	40	1 in 10,000	99.99%

”Raw data”: FASTQ format - quality line

```
TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG

@@BF#2ADHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ
```



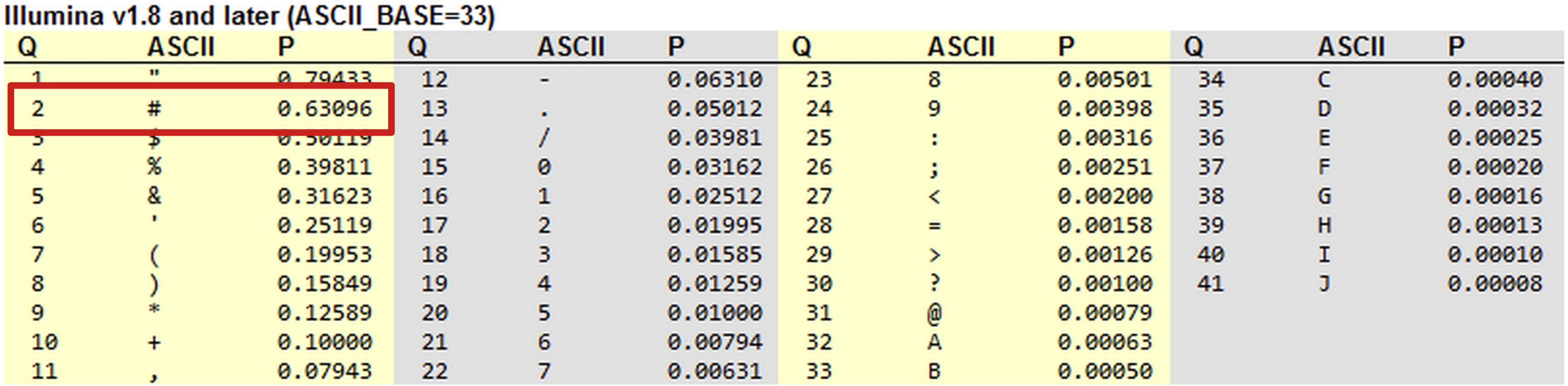
Illumina v1.8 and later (ASCII_BASE=33)

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#2ADHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ
```



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
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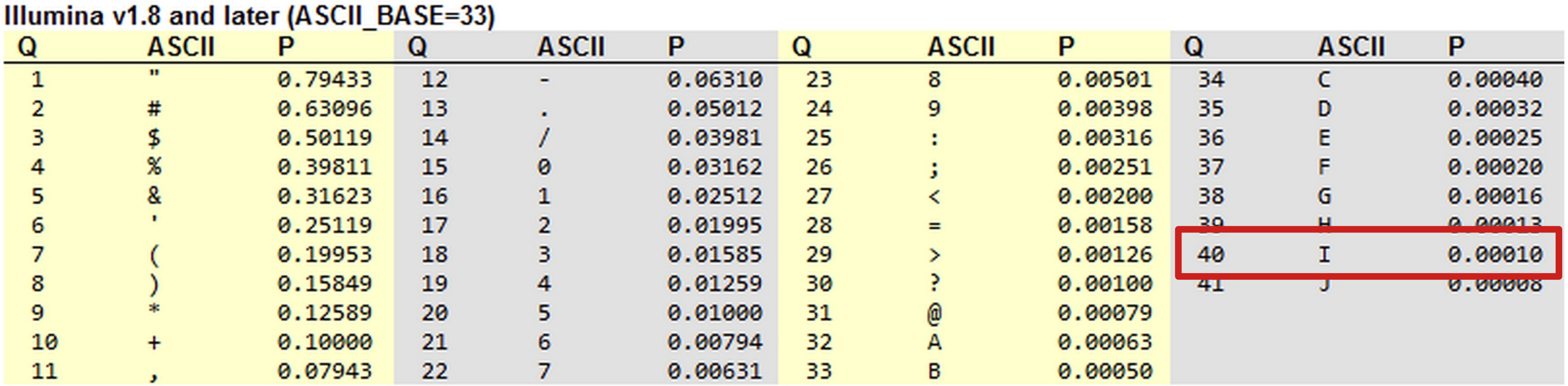
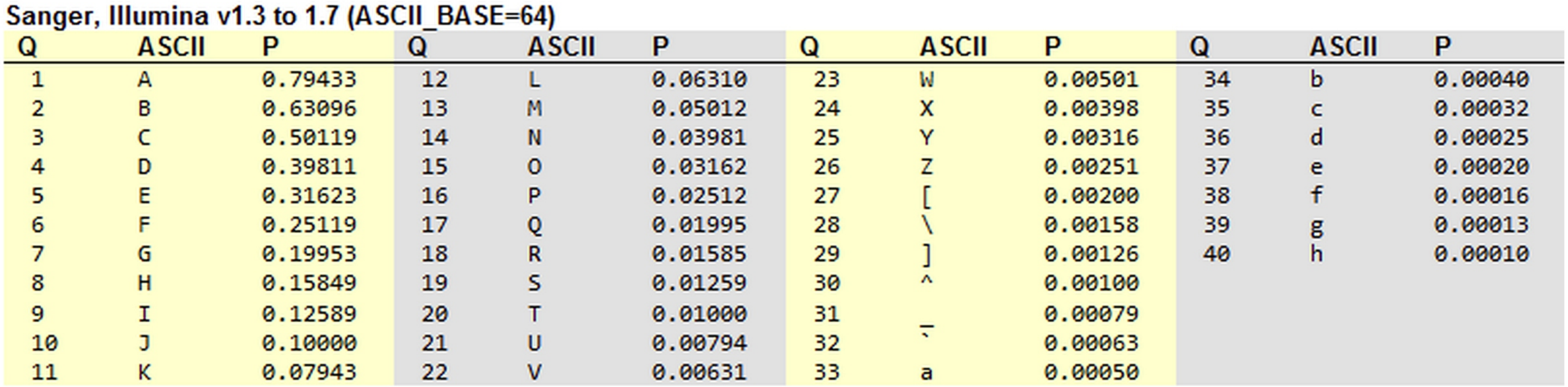
```
TCTCNAGATAAAATCAAAACCAACAGAGAGTCTAGAATAAAAGTGAATAG

@@BF#2ADHHHHHJJJJIJJJJJJIJGJIJHIIGIHIIIIIJJHIHIJJJ
```



Illumina v1.8 and later (ASCII_BASE=33)

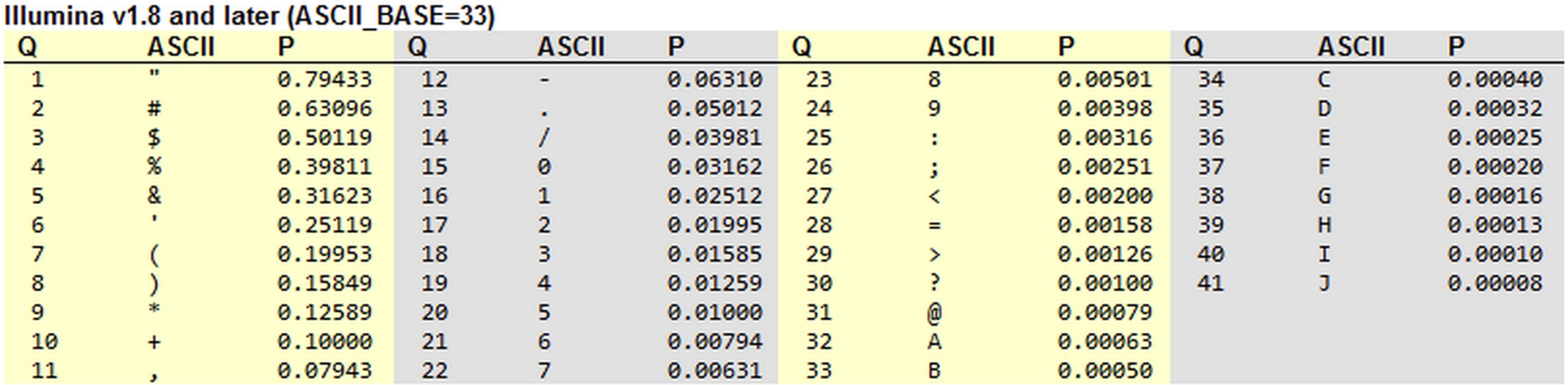
Q	ASCII	P	Q	ASCII	P	Q	ASCII	P	Q	ASCII	P
1	"	0.79433	12	-	0.06310	23	8	0.00501	34	C	0.00040
2	#	0.63096	13	.	0.05012	24	9	0.00398	35	D	0.00032
3	$	0.50119	14	/	0.03981	25	:	0.00316	36	E	0.00025
4	%	0.39811	15	0	0.03162	26	;	0.00251	37	F	0.00020
5	&	0.31623	16	1	0.02512	27	<	0.00200	38	G	0.00016
6	'	0.25119	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 using FastQC

- **Help spot problems in the sequencer or in the starting library material**

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

- **Generates an HTML report with various metrics**
  - **Average quality per position**
  - **GC% profile**
  - **Adapter presence**
  - **...**
- **input formats:** FASTQ (gzip) , SAM, BAM

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  - **GC% profile**
  - **Adapter presence**
  - **...**
- **input formats:** FASTQ (gzip) , SAM, BAM

Combining multiple reports : MultiQC

- **FastQC : 1 report for each fastq file**
- **MultiQC combine individual reports into a single report**

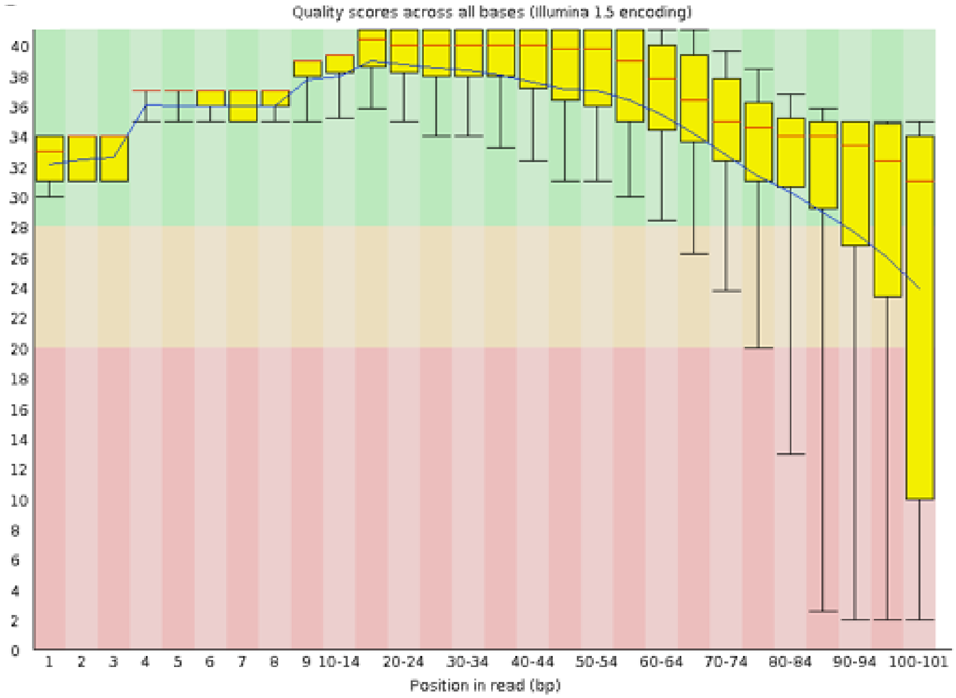
https://multiqc.info/

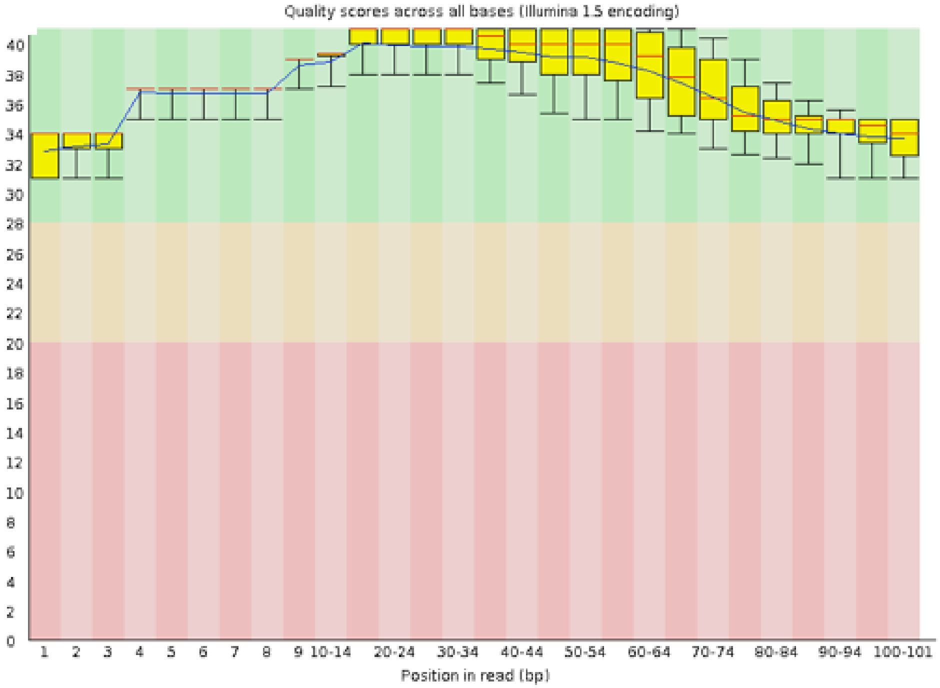
- **MultiQC also works with other tools outputs:**
  - **Trimming output**
  - **Mapping output**
  - …

PRACTICAL

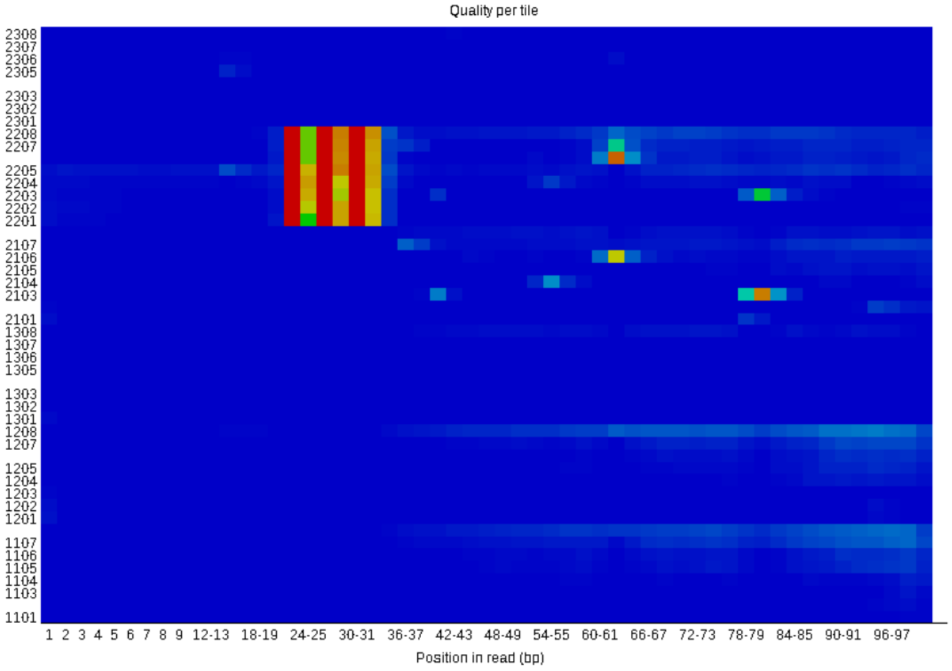
- **Go to the website and do the QC practical**

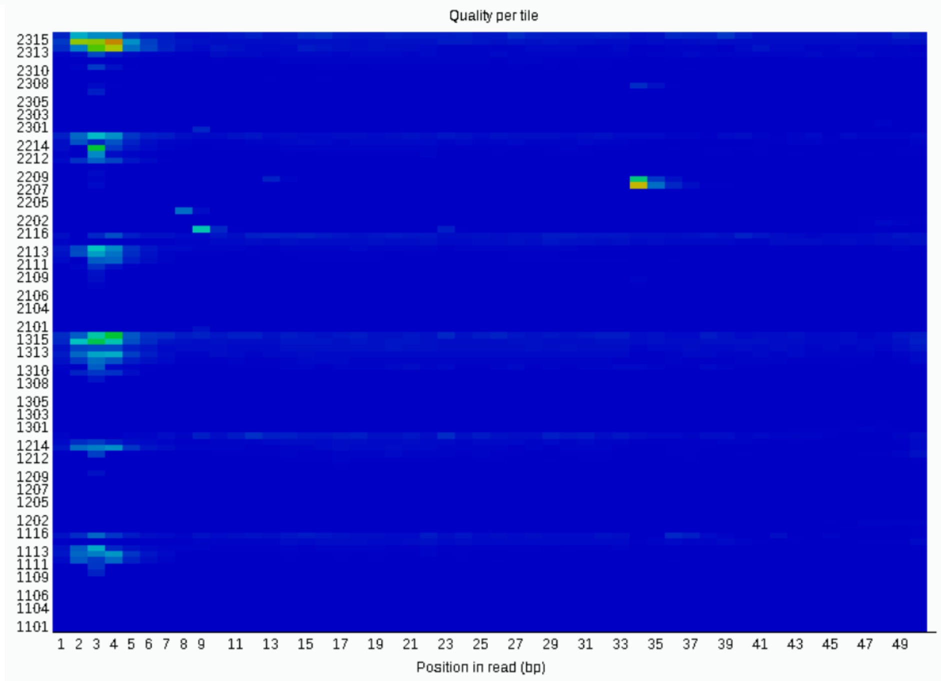
Per Base Sequence Quality





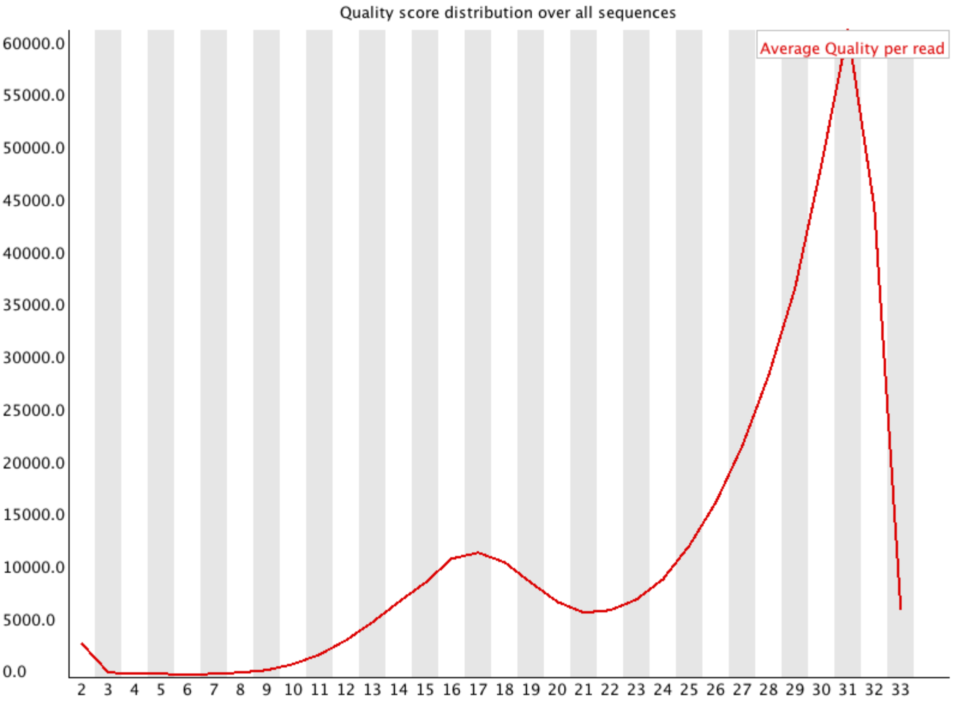
Per Tile Sequence Quality

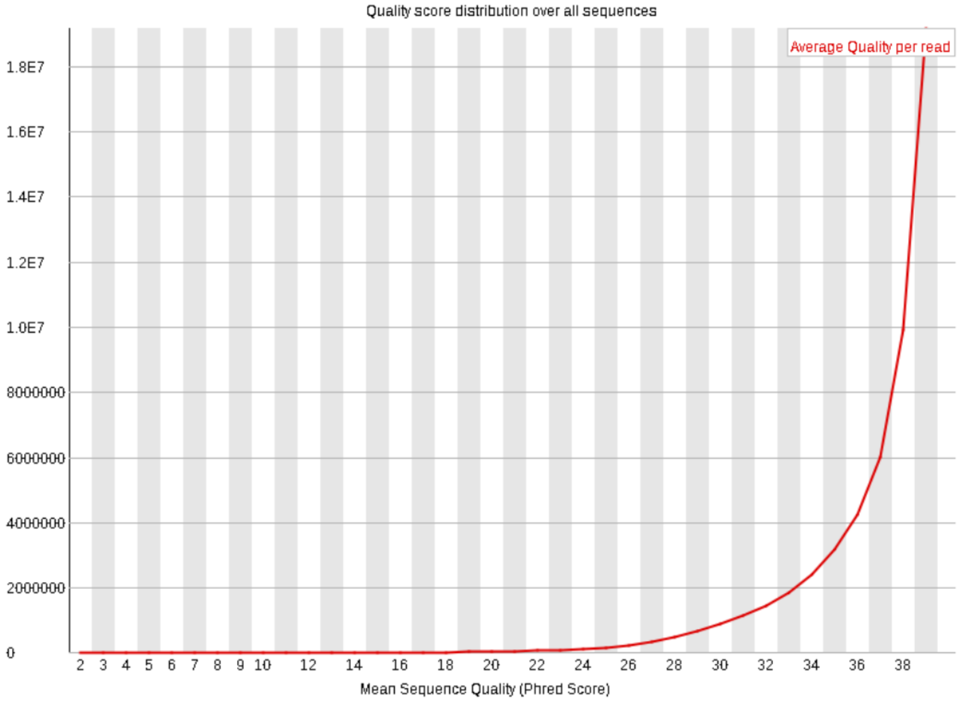




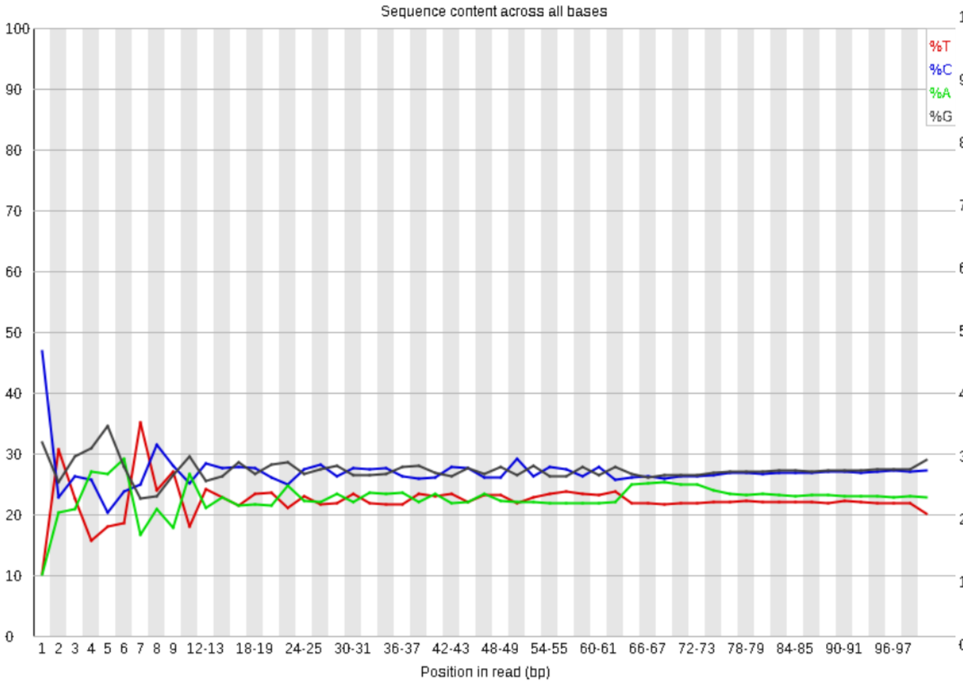
- **Only present when the fastq id contains tile id**

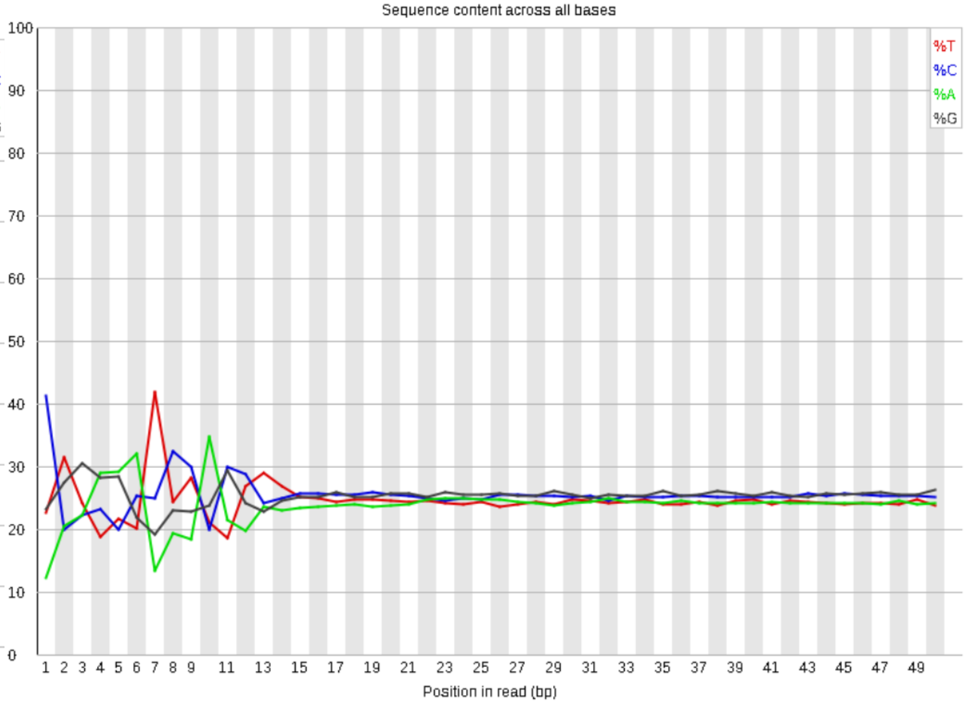
Per Sequence Quality Scores



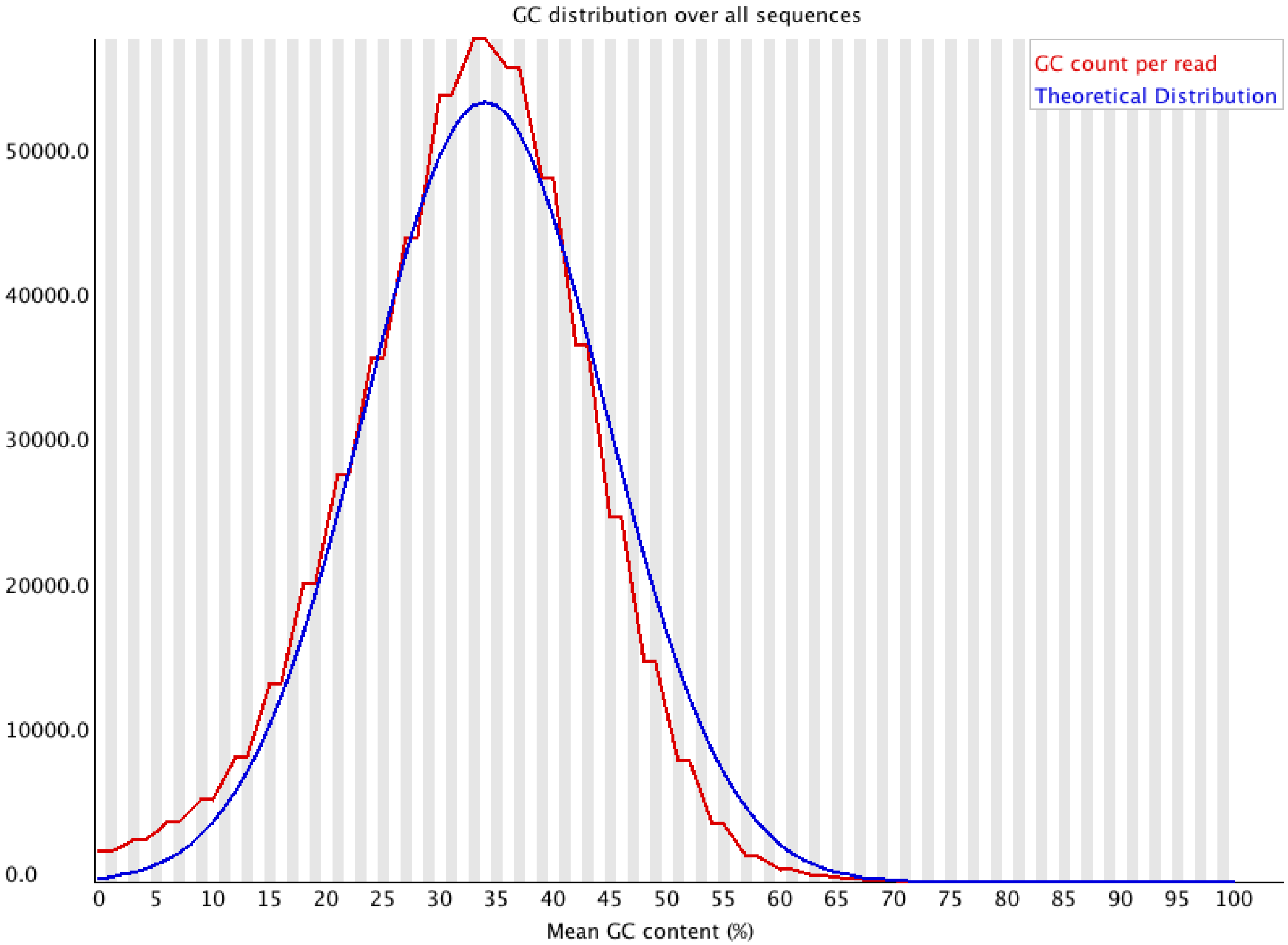


Per Base Sequence Content

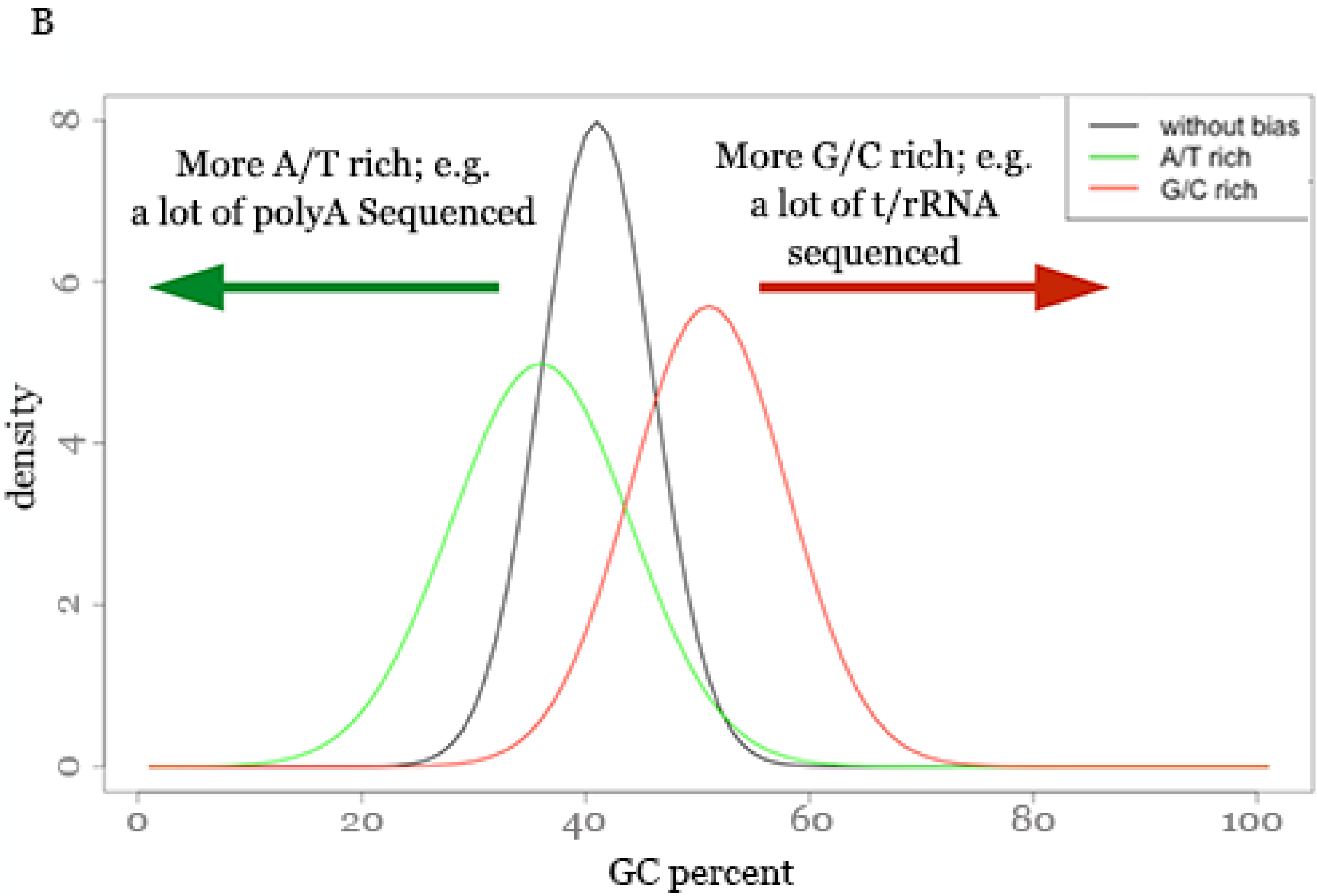




Per Sequence GC Content



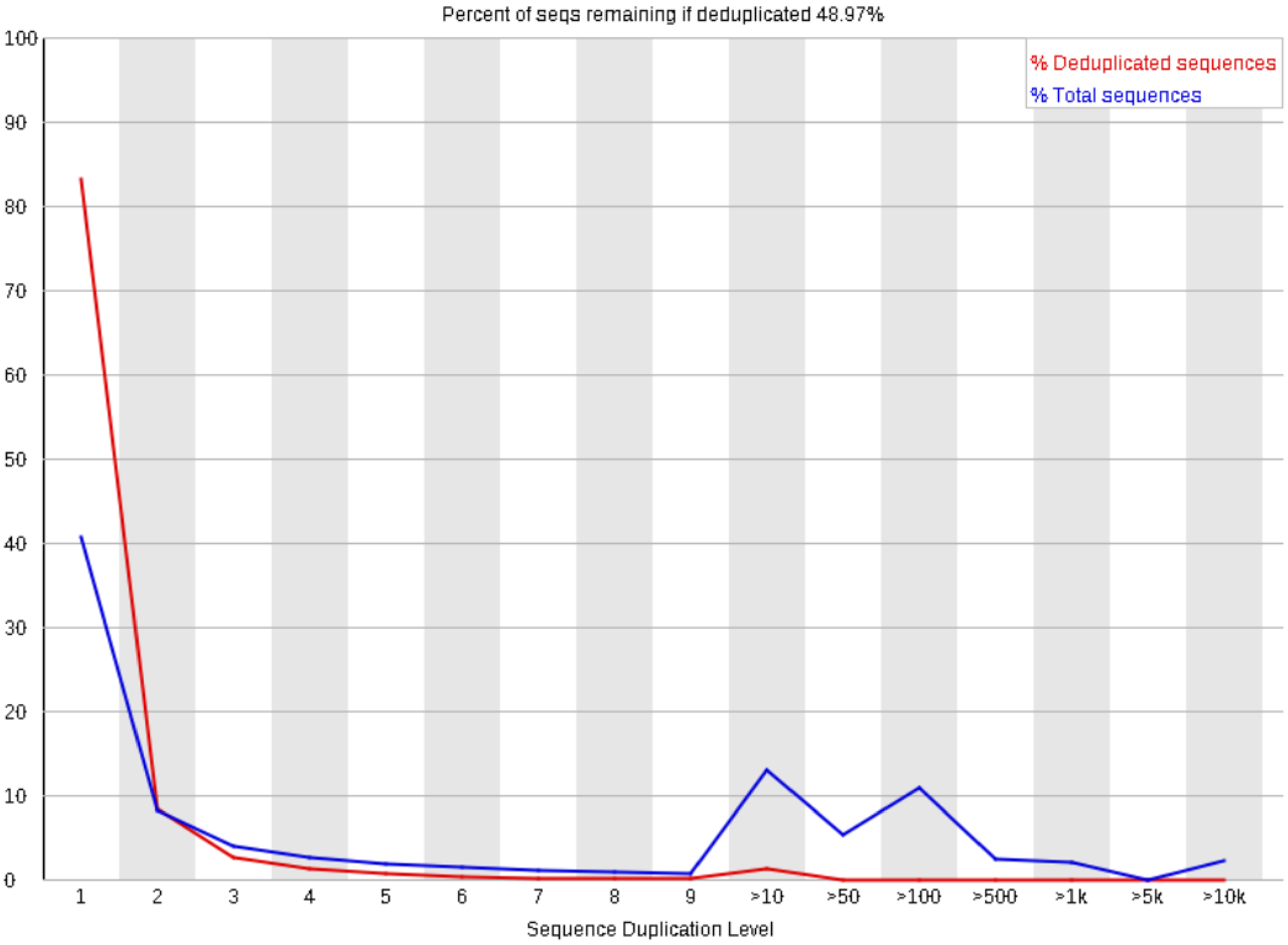
Per Sequence GC Content



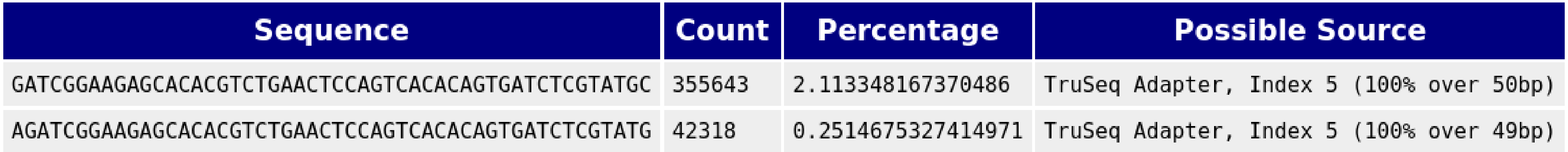
More A/T rich; e.g. a lot of polyA Sequenced

More G/C rich; e.g. a lot of t/rRNA sequenced

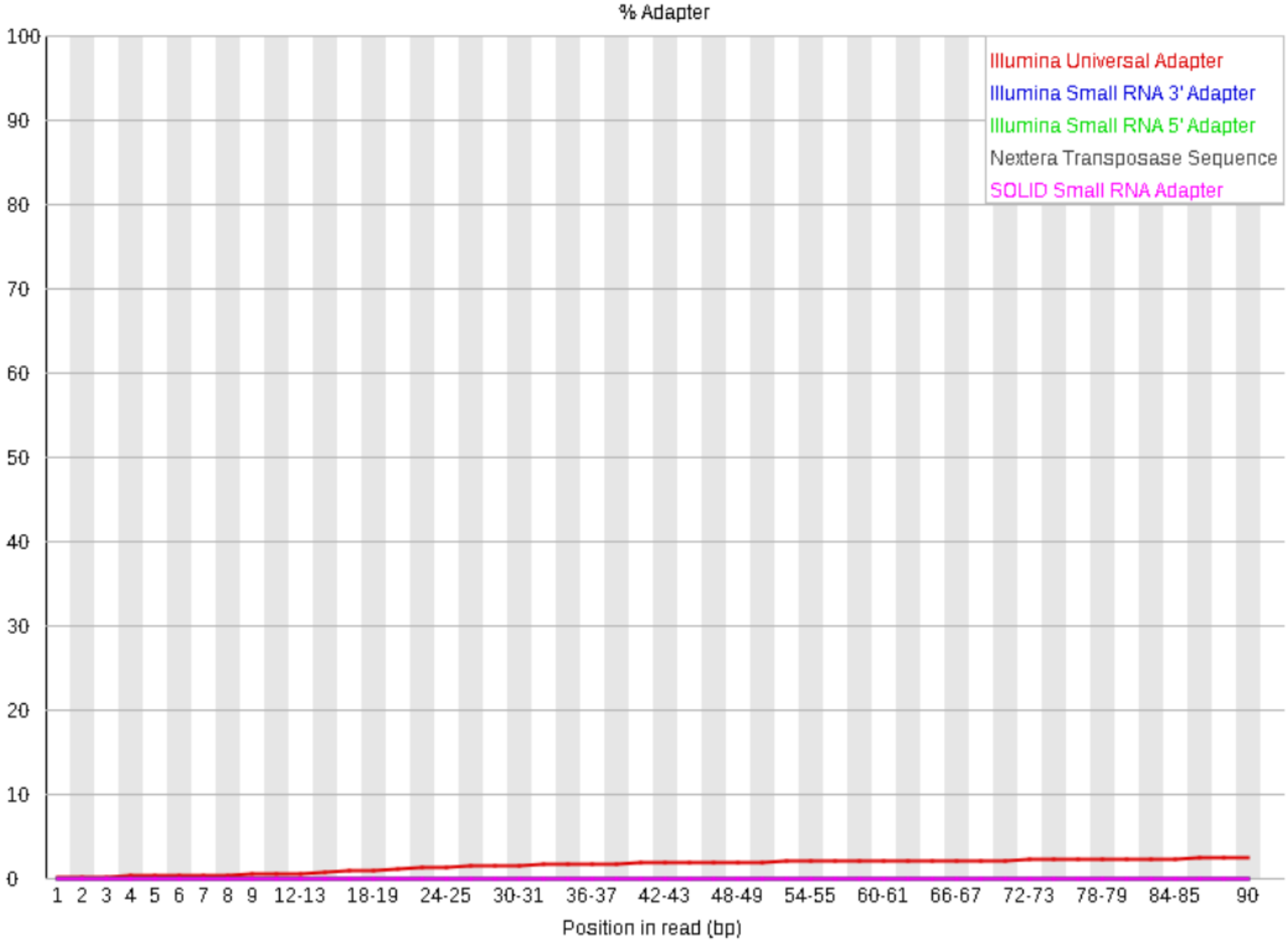
Duplicate Sequences

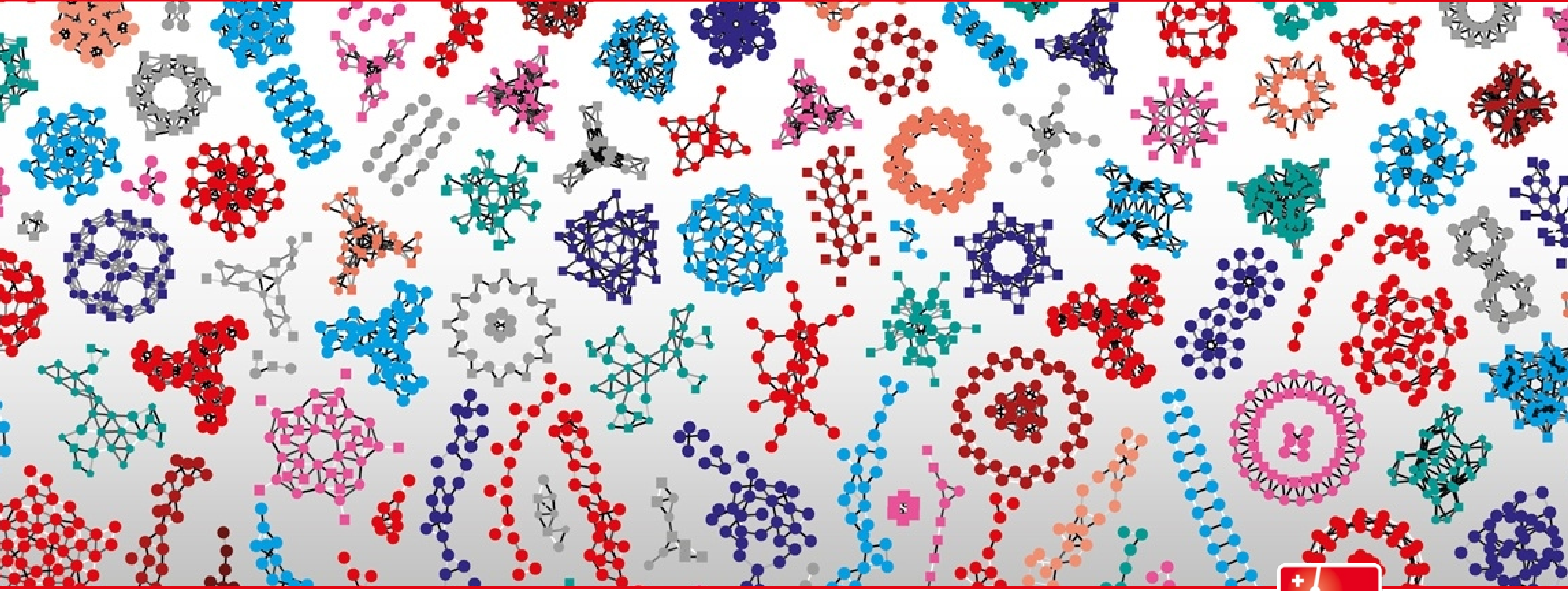


Overrepresented Sequences



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)





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Contributors:

Wandrille Duchemin
Geoffrey Fucile
Walid Gharib
Pablo Escobar Lopez
Mihaela Zavolan