Illumina Reads QC

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Sources for Illumina Reads QC

- Basic summaries in:
  - MiSeq Control Software (MCS) primary analysis: run-time statistics and predictions
  - MiSeq Reporter after secondary analysis: summaries of clusters, phasing, errors across the run

- Sequencing Analysis Viewer (SAV): very detailed (per lane, tile, cycle) in real time and post-run
  - Ideal for troubleshooting

- FastQC: open source tool, analyses reads files

Phred Scale Q score

- $Q = -10 \cdot \log_{10} P_{incorrect}$ $\Rightarrow$ $P_{incorrect} = 1 / 10^{Q/10}$
- “10 Q more is 10 times better” (as in: 10 times fewer errors)
- Q-score divided by 10 is “number of nines”

Phred Score (Q)	Probability of incorrect call	Probability of incorrect call	Base call accuracy	Characters (Phred+33)
0	$1/10^0$	1 (100%)	-	!"#$%&'()*
10	$1/10^1$	0.1 (10%)	90%	+,-./01234
13	$1/10^{1.3}$ (~1/20)	0.05 (5%)	95%	.
17	$1/10^{1.7}$ (~1/50)	0.02 (2%)	98%	2
20	$1/10^2$	0.01 (1%)	99%	56789:;<=>
30	$1/10^3$	0.001	99.9%	?@ABCDEFGH
40	$1/10^4$	0.0001	99.99%	IJKLMNOPQR
50	$1/10^5$	0.00001	99.999%	STUVWXYZ[\

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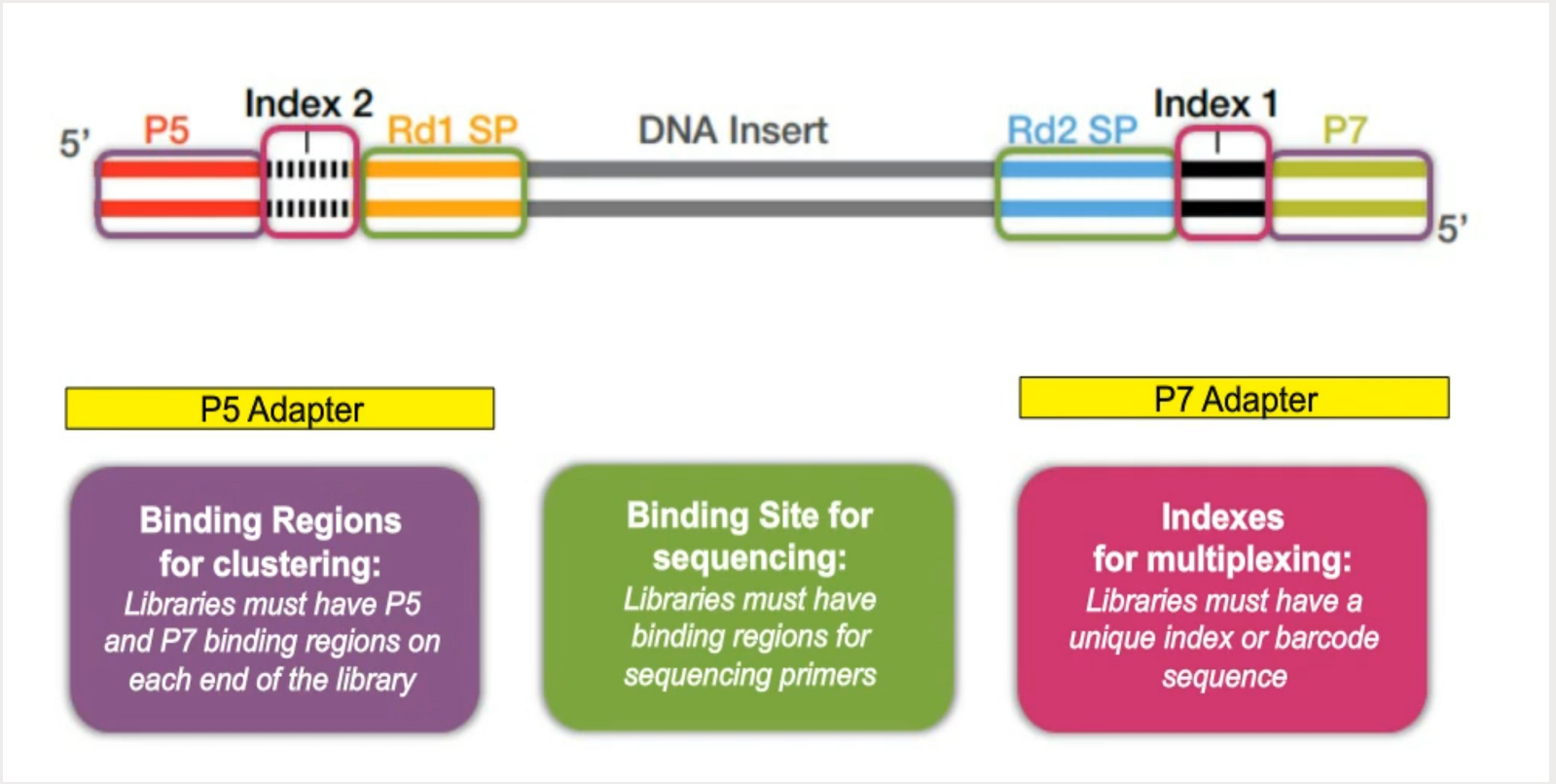
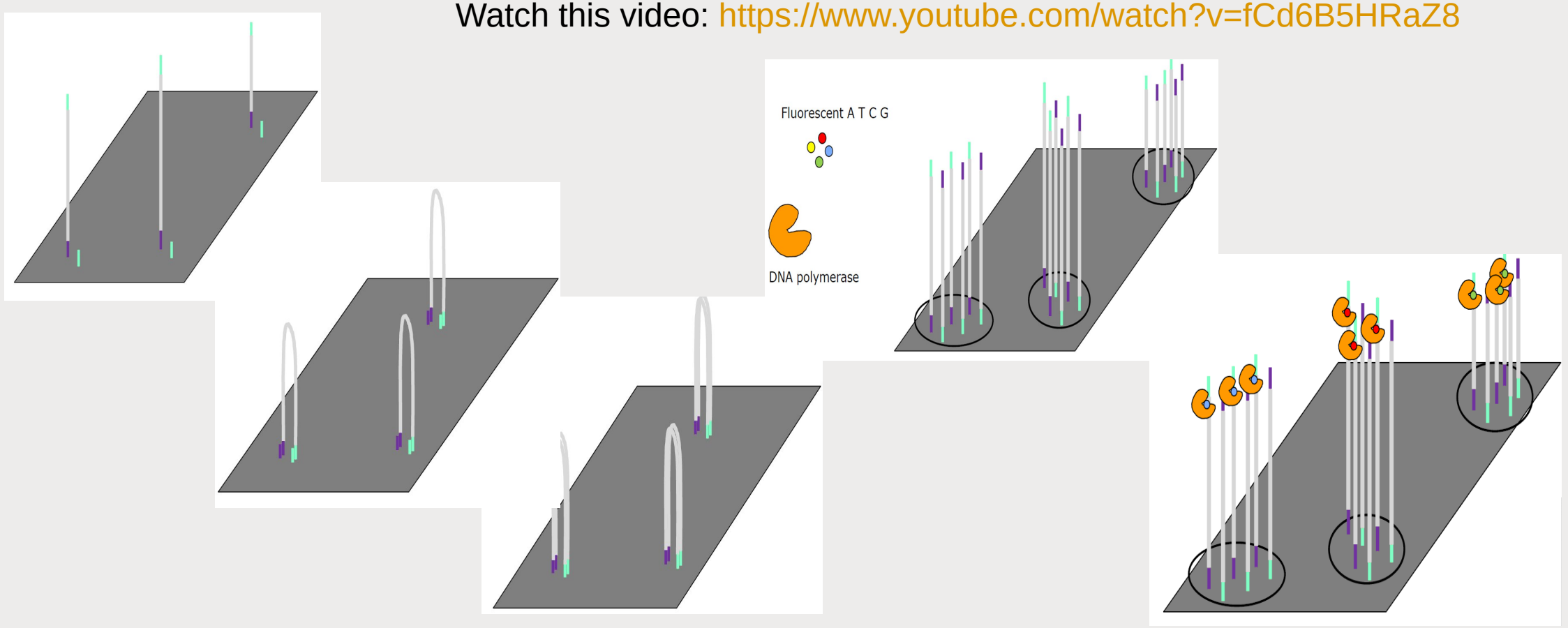
What are Adapters?



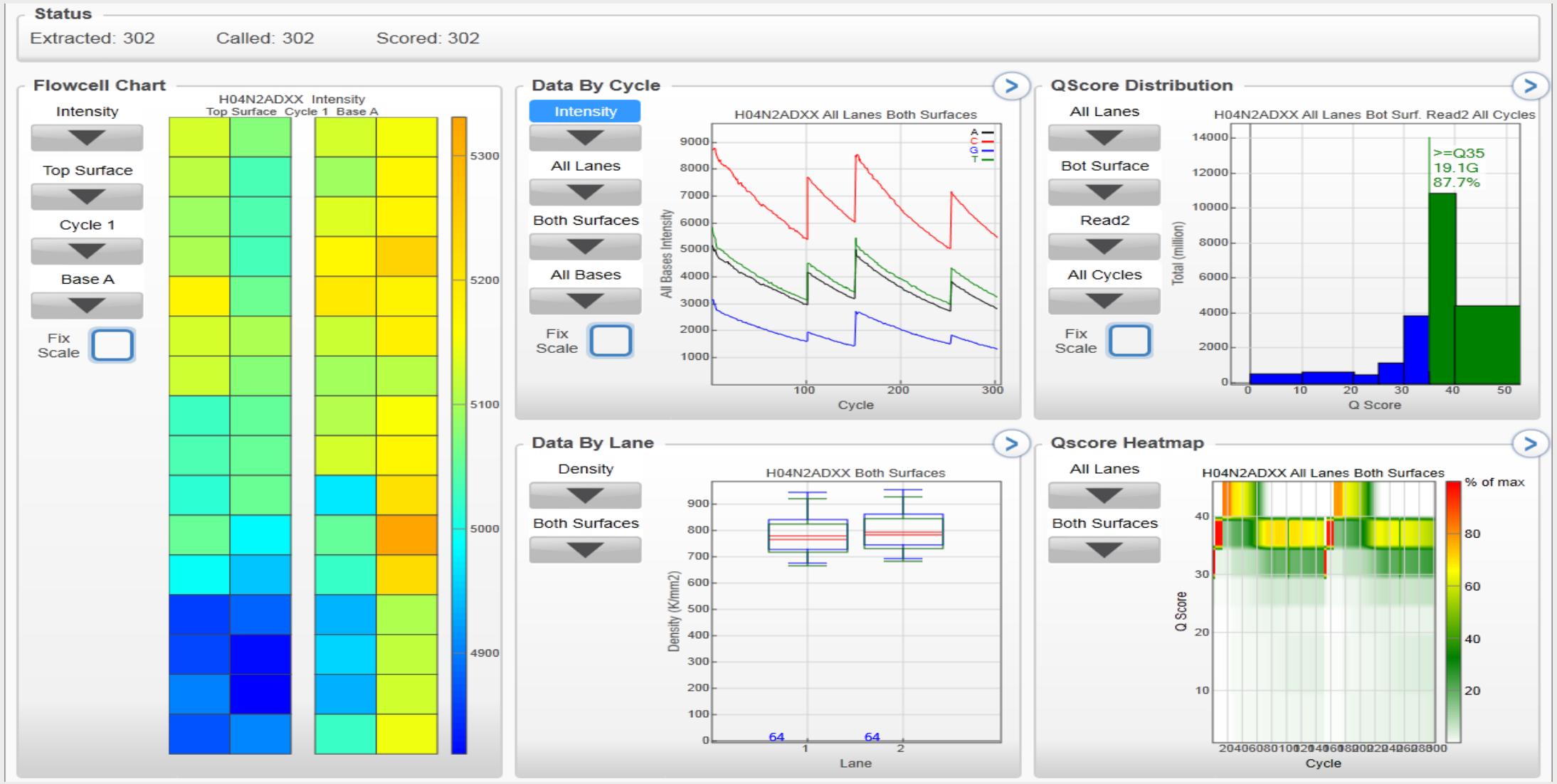
What are Clusters and Cycles?

(and lanes, tiles, and swathes?)

Watch this video: https://www.youtube.com/watch?v=fCd6B5HRaZ8



Sequencing Analysis Viewer



Sequencing Analysis Viewer

Sequencing Analysis Viewer

Run Folder: Y:\101029_P22_0759_BFC805GRAB

Browse | Refresh

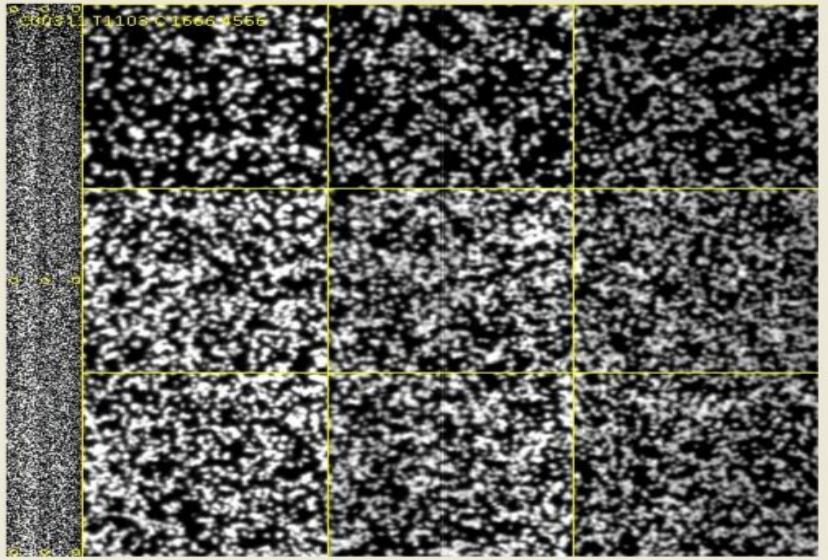
Analysis | Imaging | Summary | Tile Status | Controls

Cycle 1 | Lane 1 | Surface Top | Swath All | Section 3

A C G T

Index	Lane	Tile	Section	Cycle	Surface	Swath	Time	P90 A	P90 C	P90 G	P9
1297	1	1103	3	1	Top	1	10/29/201...	3326	3944	1729	41
1298	1	1103	3	2	Top	1	10/29/201...	3306	3906	1741	4(
1299	1	1103	3	3	Top	1	10/29/201...	3259	3853	1699	4(
1300	1	1103	3	4	Top	1	10/29/201...	3211	3806	1720	4(
1301	1	1103	3	5	Top	1	10/30/201...	3087	3703	1691	37
1302	1	1103	3	6	Top	1	10/30/201...	3066	3677	1610	35
1303	1	1103	3	7	Top	1	10/30/201...	2959	3554	1577	35
1304	1	1103	3	8	Top	1	10/30/201...	2945	3547	1558	35
1305	1	1103	3	9	Top	1	10/30/201...	2918	3518	1548	35
1306	1	1103	3	10	Top	1	10/30/201...	2906	3491	1536	35
1307	1	1103	3	11	Top	1	10/30/201...	2881	3461	1512	34
1308	1	1103	3	12	Top	1	10/30/201...	2869	3452	1498	34
1309	1	1103	3	13	Top	1	10/30/201...	2835	3423	1510	35
1310	1	1103	3	14	Top	1	10/30/201...	2854	3443	1493	34
1311	1	1103	3	15	Top	1	10/30/201...	2857	3445	1482	34
1312	1	1103	3	16	Top	1	10/30/201...	2811	3392	1452	33
1313	1	1103	3	17	Top	1	10/30/201...	2779	3356	1471	34
1314	1	1103	3	18	Top	1	10/30/201...	2756	3338	1435	33
1315	1	1103	3	19	Top	1	10/30/201...	2778	3364	1419	32
1316	1	1103	3	20	Top	1	10/30/201...	2749	3335	1435	33
1317	1	1103	3	21	Top	1	10/30/201...	2740	3324	1434	33
1318	1	1103	3	22	Top	1	10/30/201...	2692	3271	1441	33
1319	1	1103	3	23	Top	1	10/30/201...	2721	3308	1423	33
1320	1	1103	3	24	Top	1	10/30/201...	2687	3270	1420	33
1321	1	1103	3	25	Top	1	10/30/201...	2656	3229	1398	32

Rows=20736 | Disp=162 | Sel=1 | Filter



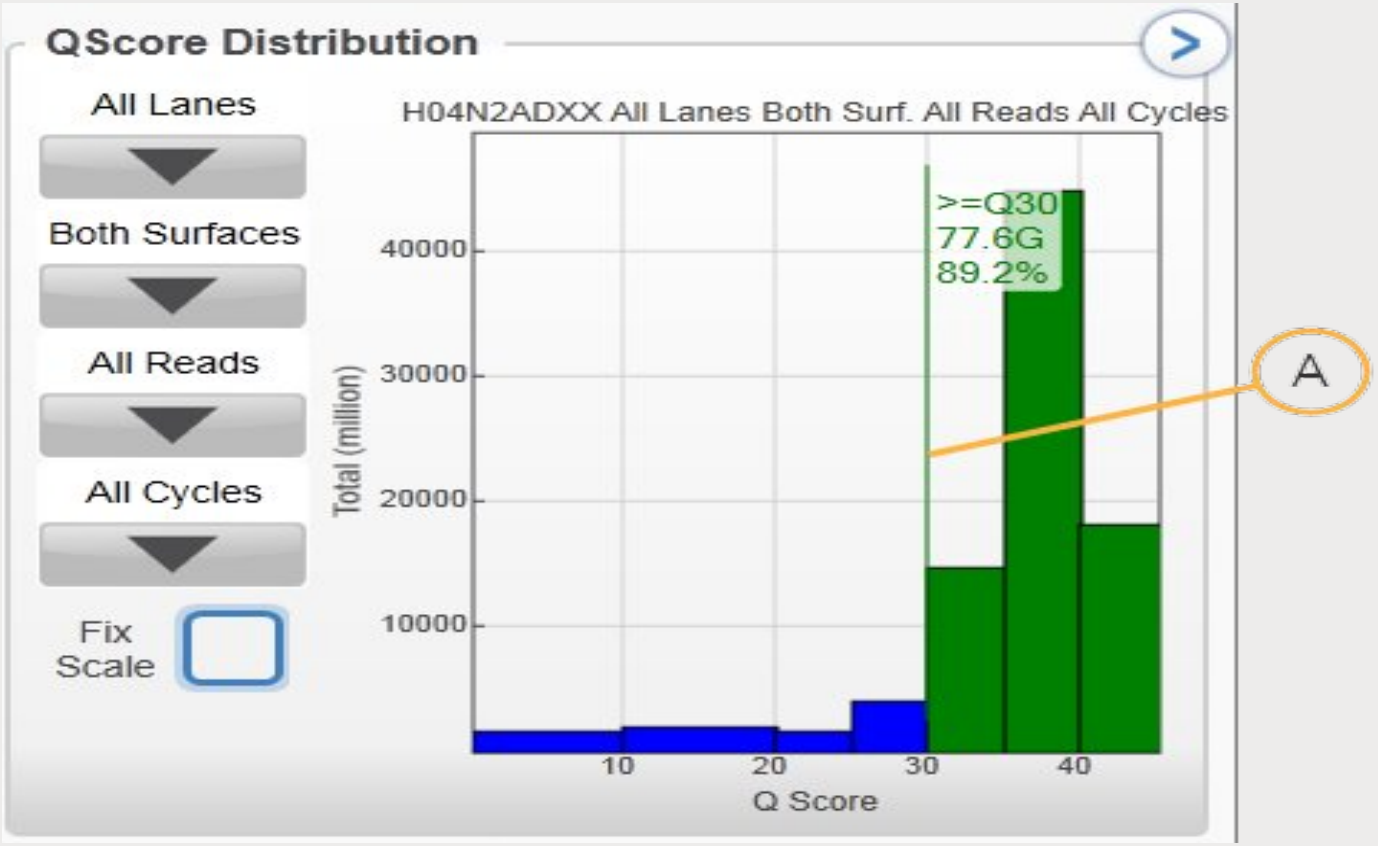
Main Metrics

- Yield (# bases): total number of bases read
- Error rate (%): percentage called incorrectly
- Q30+ (%): percentage bases with Q30+ score
- Density (K/mm^2): thousands of clusters per mm^2
- Clusters PF (%): percentage passing chastity filter
- (Pre-)phasing (%): ‘jumping’ or lagging bases

Metric: Error Rate

- Percentage of bases called incorrectly
- How does it know? PhiX
- Also:
  - % Aligned to PhiX (should match spiked %)
  - % Perfect reads (relative to PhiX)

Metric: Percentage Q30+



Metric: (Pre-)Phasing

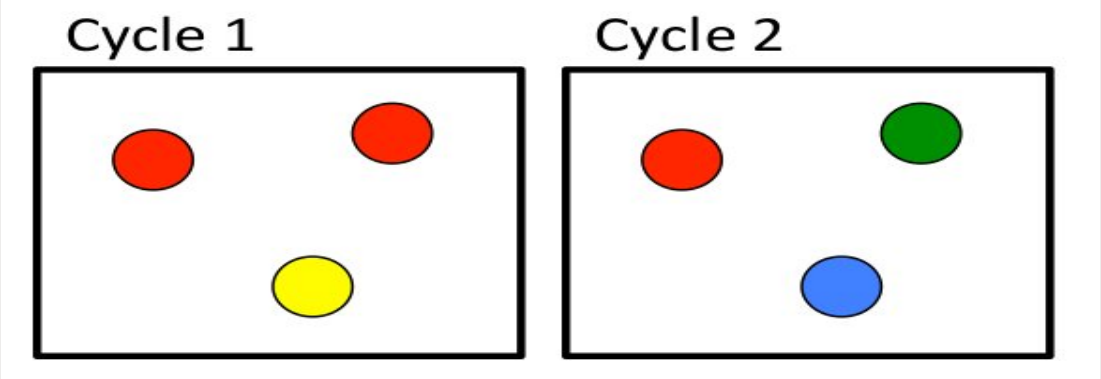
- Percentage of bases in cluster lagging or ‘jumping ahead’
  - Phasing: no base attaches, sequencing falls behind
  - Pre-phasing: skipping a base, sequencing jumps ahead
- Sequencer corrects for this (to some extent)
- Rule of thumb: below 0.5%, preferably <0.1%
- Causes: unbalanced bases, reagents or flow cell quality, temperature

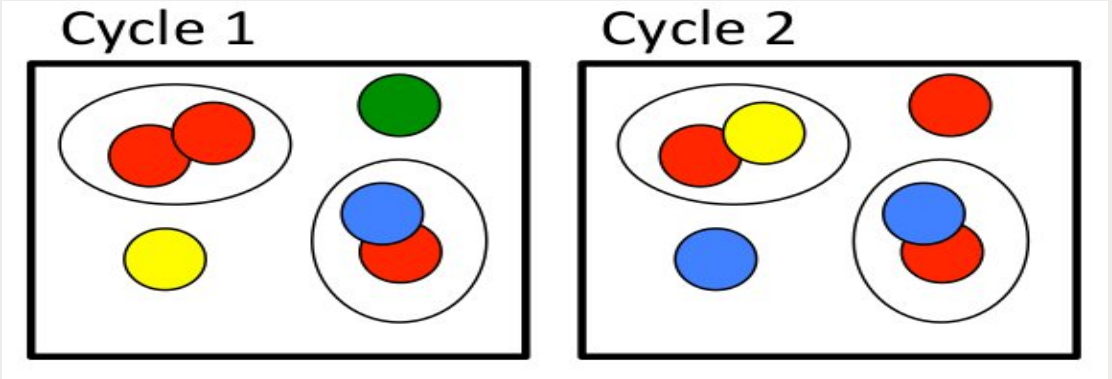
Metric: Clusters PF (passing filter)

- Chastity value:
  - Intensity of called base divided by sum of called base and second brightest
  - Threshold value is 0.6 (so must be “50% better than” second brightest base)
- Passing rule:
  - In first 25 cycles at most 1 base may have chastity under 0.6
- Subsequent SAV statistics are for Clusters PF
- Rule of thumb: 80% or higher

Metric: Cluster Density

- Bases hard to call
  - Q values down, %Error up
  - %PF and yield decrease
  - Index read failures
- Reverse read even worse
- Low diversity exacerbates
  - Early cycles determine cluster locations
  - Spike in more PhiX
- Underclustering: intensity and focus issues





FastQC Outputs

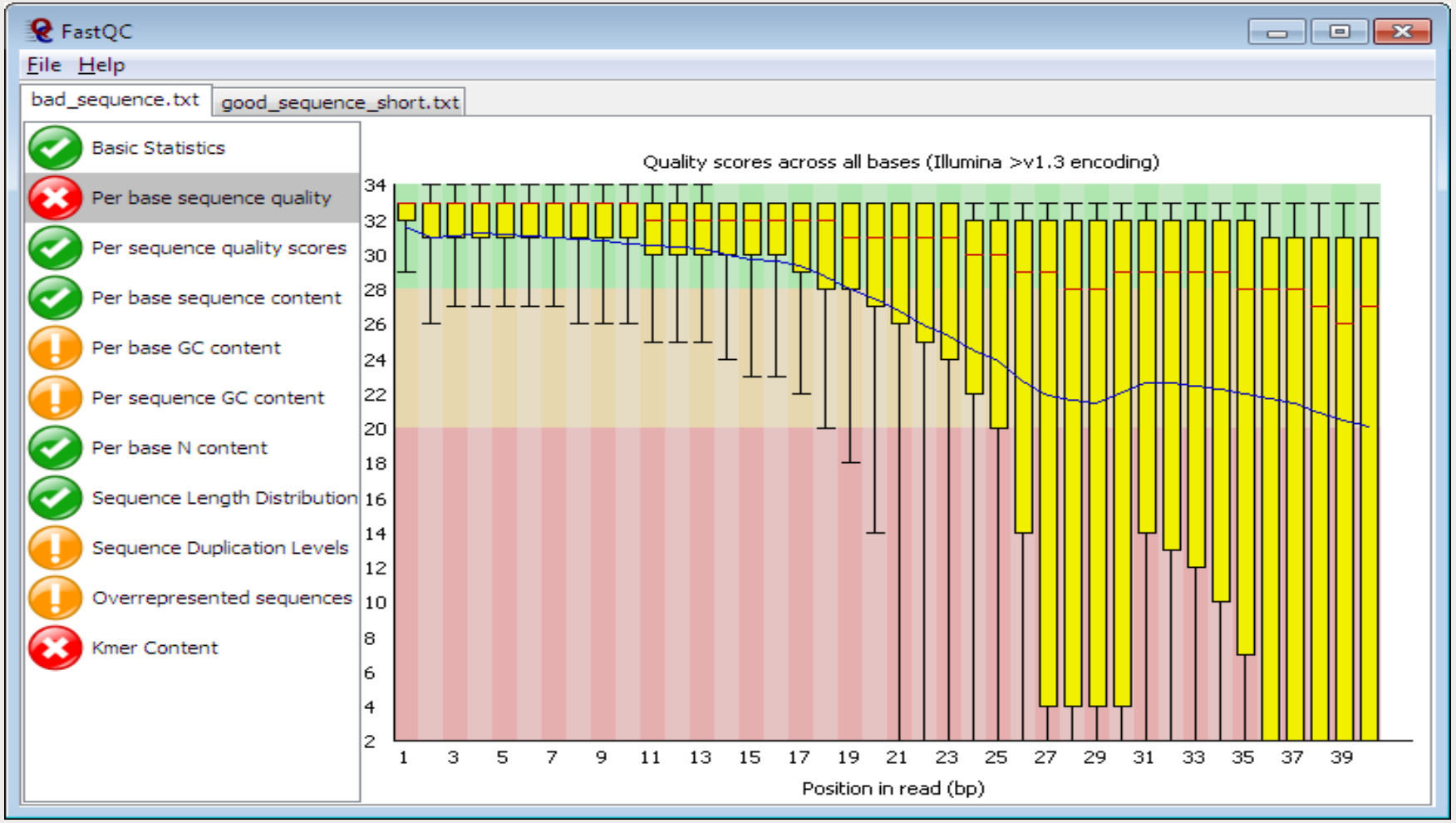
- http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
- https://sequencing.qcfail.com/

FastQC: Basic Stats

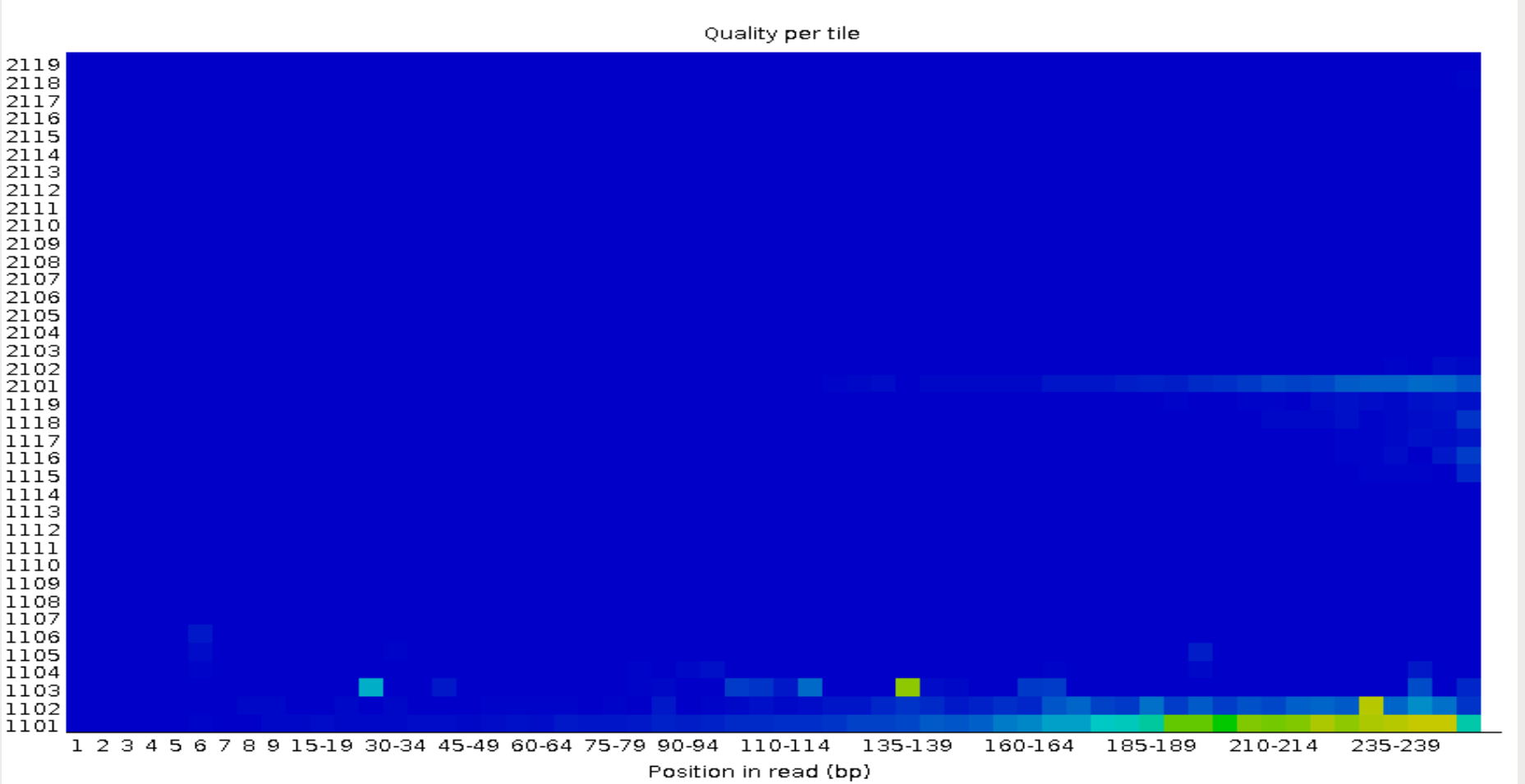
Basic Statistics

Measure	Value
Filename	110LF_R1.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	743046
Sequences flagged as poor quality	0
Sequence length	35-251
%GC	56

FastQC: per base sequence quality



FastQC: Per tile (and cycle) quality



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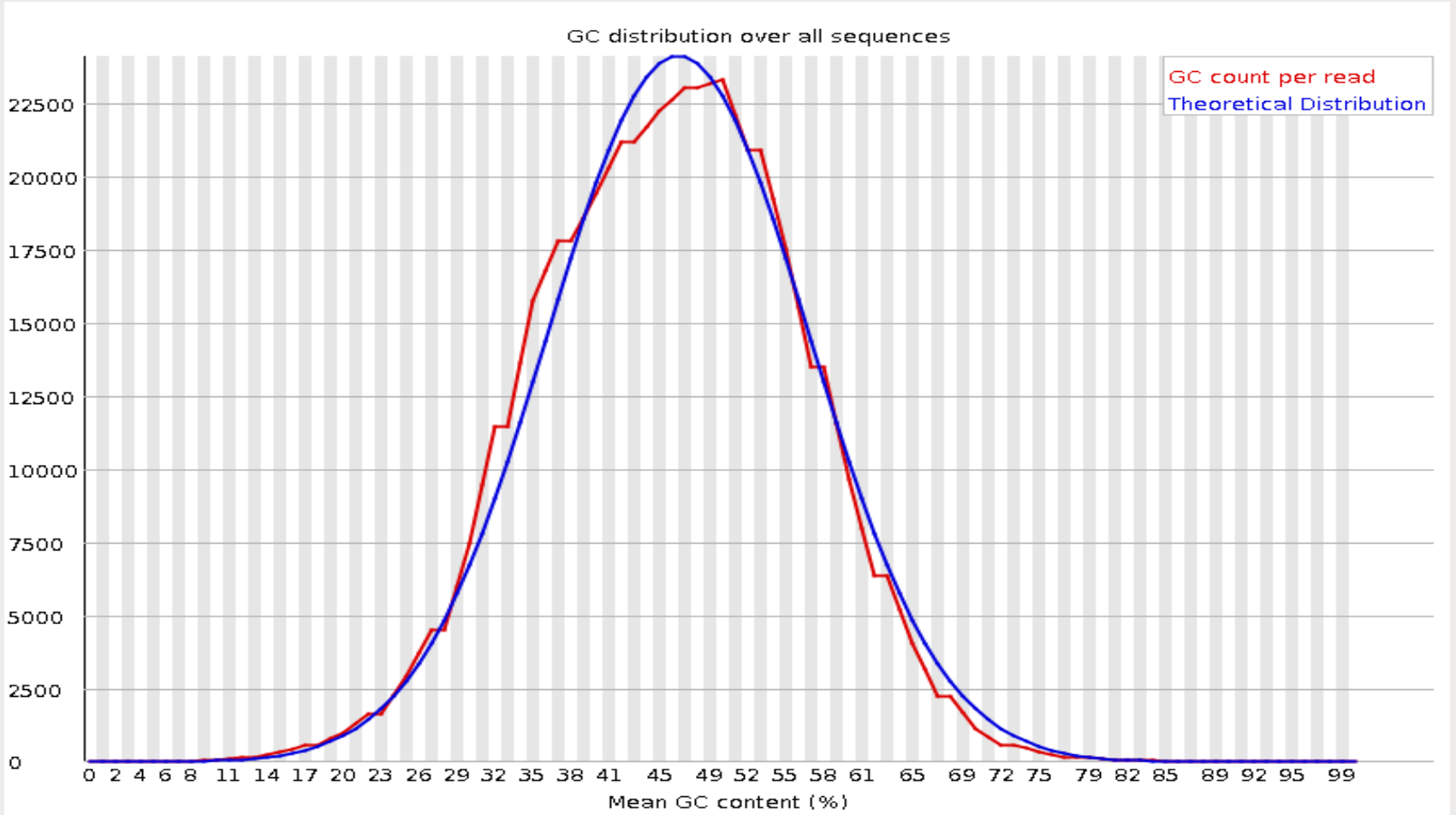
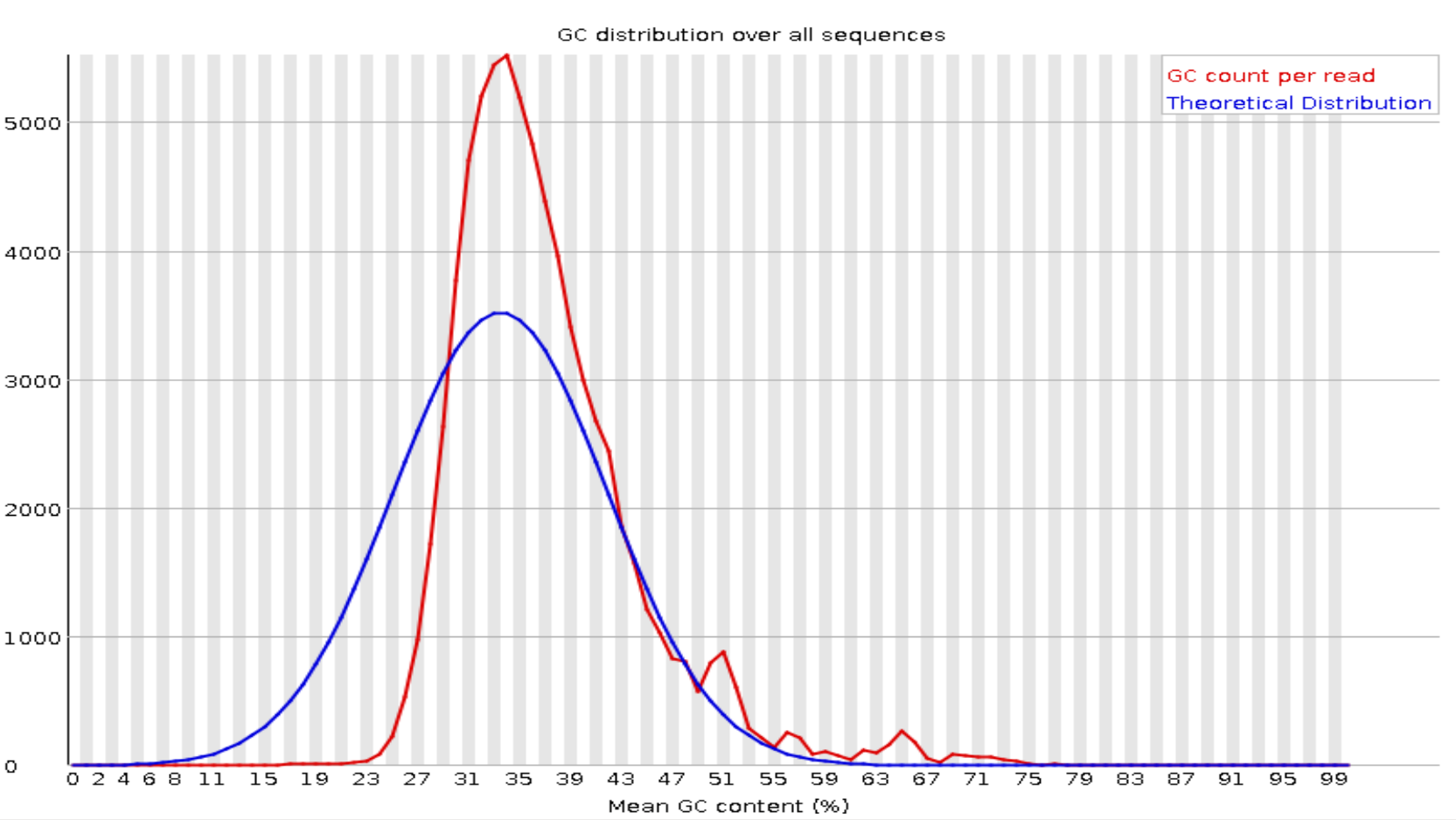
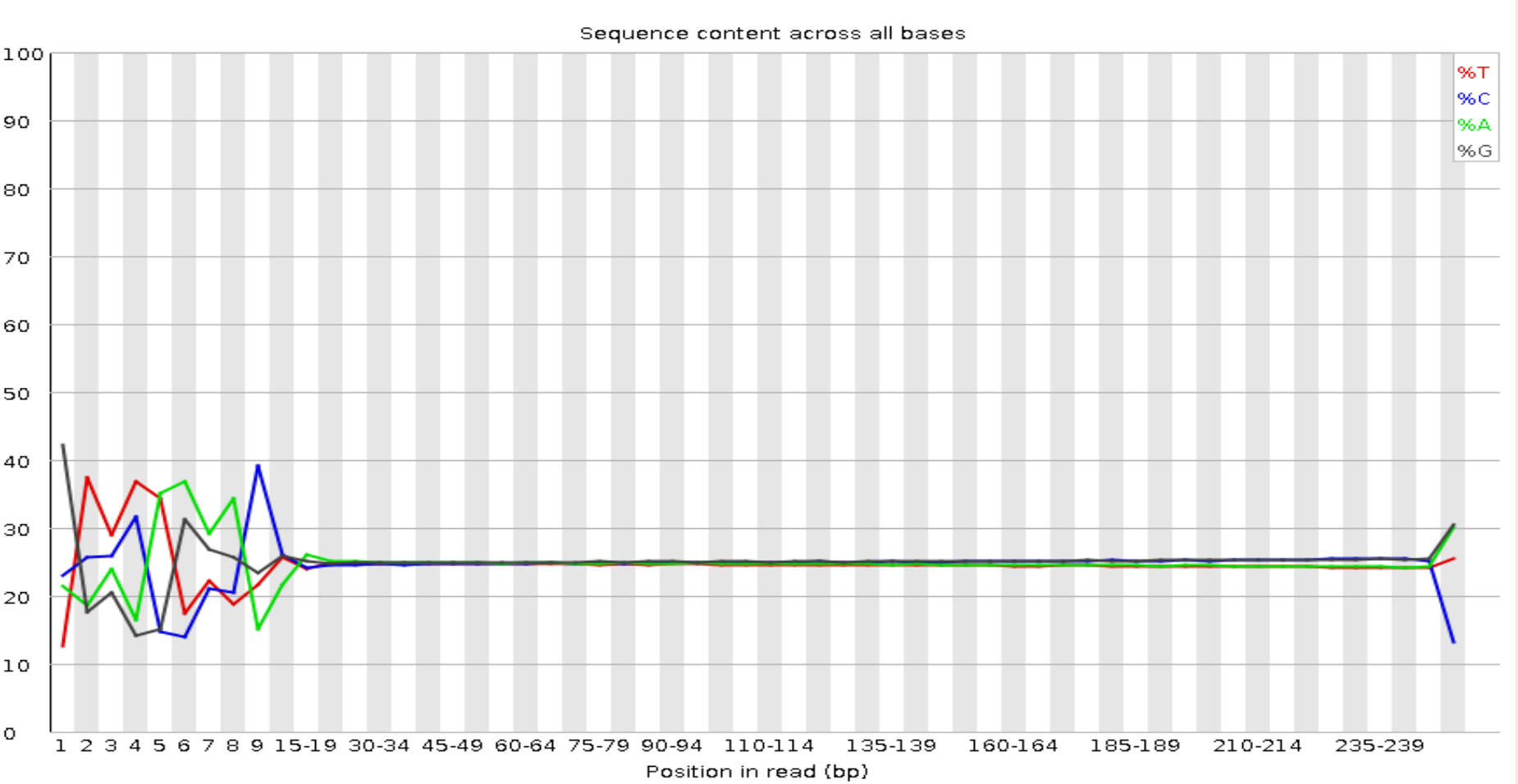
FastQC: GC distribution



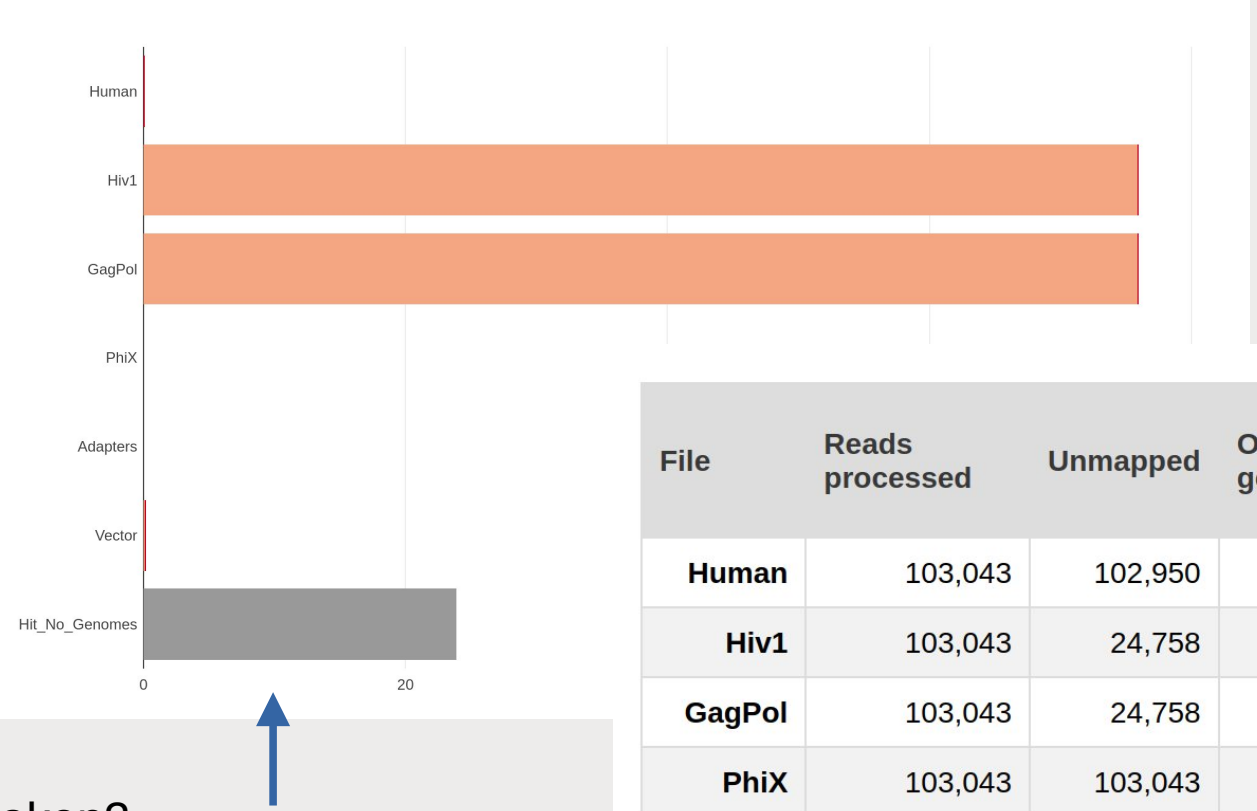
FastQC: unlikely GC distribution



FastQC: proportions of bases



Bonus: FastqScreen



File	Reads processed	Unmapped	One hit / one genome	Multiple hits / one genome	One hit / multiple genomes	Multiple hits / multiple genomes
Human	103,043	102,950	52	23	5	13
Hiv1	103,043	24,758	0	0	78,262	23
GagPol	103,043	24,758	0	0	78,262	23
PhiX	103,043	103,043	0	0	0	0
Adapters	103,043	103,043	0	0	0	0
Vector	103,043	102,903	50	4	72	14

Kraken2
(metagenomic binner)
may actually be more useful!

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Thank you

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